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SIGNIFICANT RÔLES OF TRACE ELEMENTS IN THE NUTRITION OF PLANTS[1, 2]

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During the past quarter of a century much has been written concerning the place of the so-called trace elements in the economy of plant life. Most of this literature deals with the effects produced upon plants when these elements are absent from the growth substrate and is purely descriptive in nature, portraying the symptoms of deficiency. There is also, in the physiological literature of the past decade and a half, much descriptive material portraying the pathological symptoms which appear in plants when these elements occur in the plant environment in concentrations in excess of those required for growth and development. Thus far these qualitative observations make up practically all of the great mass of the investigational literature upon this subject. So much of this foundation in natural fact has already been accomplished, that intensive activity along this line of categorical description can no longer be considered an adequate field of investigation in this particular phase of plant nutrition. It should be remembered, however, that the extension of our knowledge in this phase of nutrition cannot progress without at least some continued activity along these descriptive lines; but our chief interest is no longer in the qualitative pathological symptoms themselves, which develop as the result of trace element malnutrition, but in their relation to those more basic phenomena which can be evaluated only through careful quantitative experimental investigation. From almost the beginning of the present century studies have been directed toward ascertaining whether or not certain elements, which are found in trace quantities in both the plant and in its environment, might be included in the category of essential factors for growth and development. There is much contradictory evidence, as might be expected, relative to this question, which

[1] Journal series paper of the New Jersey Agricultural Experiment Station, Department of Plant Physiology.

[2] The sixth STEPHEN HALES address, read before the American Society of Plant Physiologists at Philadelphia, December 30, 1940.

is an important one. The energetic search for such elements which began early in the century is still in progress. It has been in the past a profitable field of investigation and it is predictable that it will provide an equally profitable field of study in the future. The inability of experimenters to grow plants successfully through a complete cycle in artificial culture, in the early years of the present century, initiated a search for missing essential factors. The usual experimental technique which had long been employed in solution culture and in sand culture was too crude, however, and the materials used were too unrefined to disclose the need of specific elements required in minute quantities in nutrient substrates for plants.

Search for the missing factors, therefore, led to great improvements in experimental techniques. Great strides have been made in the refinement of methods and in the purification of nutritive materials for accurate study in the endeavor to demonstrate the need of these elements in the nutrient substrate and to prove their essentiality. Through this refinement of technique, improvement in the methods of operation, and purification of nutritive material, it is now possible to add to the previous list of essential elements for plants boron, manganese, zinc, and copper. Undoubtedly other elements will be added to this list with still further purification of chemical materials and further refinements of physical equipment and methods of procedure. The degree of refinement at present attainable, however, imposes a rigid limitation upon experimental accuracy and this is particularly true with respect to the analytical data from quantitative studies in the field of trace element nutrition. The suggestion has recently been made that an element may not be regarded as non-essential if it is present in a nutrient substrate in concentrations greater than one part in a billion (**10**). Such a degree of purity may not be attainable at the present time. It is this point, as well as that relating to the absolute importance of the trace elements in nutrient substrates, which has recently been so strongly emphasized in connection with the development and refinement of methods for growing isolated plant tissues *in vitro* (**12, 13**). It appears that the degree of purity and the state of refinement are determining factors in any attempt to establish proof of the essentiality of a given element which is present in the plant environment in very minute concentration. It is not so simple a matter to differentiate between that which merely stimulates growth and that which is essential for growth. The fact that the presence of a minute trace of a given element in a nutrient substrate may stimulate growth of the plant does not constitute proof of essentiality, nor does failure to stimulate constitute proof that an element is not essential. In order to provide proof of the essentiality of a given element it is necessary to show that a plant cannot complete its cycle in the absence of a measurable concentration of the element in question.

In order to obtain quantitative information concerning the place which the trace elements occupy in the nutrition of plants and the rôles which they play in metabolic processes, elaborate precautions must be taken to eliminate all possible sources of contamination derived from the experimental set-up, and careful attention must be given to such matters as the purity of salts, distilled water, and cleanliness of the apparatus involved. These precautions are indispensable if the objectives of quantitative research in this field are to be attained. With the discoveries made during recent years, and with the refinement of the experimental method, it has now become a simple procedure to demonstrate the effect of deficiencies of boron, manganese, and zinc with many species of plants under the conditions of control which characterize the usual routine of solution culture experimentation.

While superficial descriptions relating to the effects of trace element deficiencies upon the various species continue to accumulate, as is to be expected, it is most encouraging and even inspiring, that a pronounced change toward the more quantitative type of investigation has been in progress during the past several years and some very important quantitative work dealing with trace element nutrition has appeared in the literature (**2, 5, 7, 8**). Difficult as it has been to demonstrate beyond a doubt that boron, manganese, and other trace elements are essential for plants, it may, however, be infinitely more difficult to prove why they are essential. But the present status of trace element nutrition in plants makes it unnecessary to dwell upon either the economic or the theoretical importance of the elements required by plants in only very minute amounts. The economic importance of these elements is obvious in view of the host of plant diseases, many of which are very destructive, which have been traced either directly or indirectly to deficiency or excess of one or more of these elements in the soils of many agricultural districts. It is to be emphasized also that not one of the many problems relating to the important rôles which these elements play in the metabolic processes of plants, has yet completely emerged from the theoretical category to take its place in the realm of fact established through experimental study. The importance of theoretical consideration of such problems is, therefore, apparent.

It has early been pointed out by several investigators that the pathological symptoms produced in plants by boron deficiency are remarkably similar to those produced by calcium deficiency under like experimental conditions. The similarity has been recognized in virtually all of the species thus far studied. This has suggested the possibility that boron is in some mysterious manner involved in those processes in the plant in which calcium is a direct reactant. This suggestion is supported by the well-known fact that both boron and calcium are especially effective in the intensive metabolic processes which are confined largely to the meristematic regions, and recent investiga-

tions have shown also an intensive utilization of boron by the reproductive organs of the plant (**6**).

Attention will now be directed to the consideration of quantitative experimental evidence relating to an important rôle which boron plays in the accumulation, assimilation, and metabolism of calcium in several species widely separated in their morphological, physiological, and taxonomic characteristics. But, before presenting this evidence it will be necessary to describe very briefly, the experimental procedure by which these data were obtained. Relatively large numbers of plants of each species dealt with were grown in a standard culture medium until they had attained the age and phase of growth and development desired before the experimental treatments were begun. At the beginning of the treatment period the cultures were divided into three groups or series: The cultures of the first series covered a range of boron concentrations in the standard nutrient substrate which, by previous tests, was known to produce visible symptoms of deficiency in the species in question. The cultures of the second series covered a range of approximately optimum boron concentrations; and the cultures of the third series covered a range of boron concentrations sufficiently high to produce symptoms of toxicity in the plants of the species in question. With the exception of the boron treatments the nutrient substrate used in the cultures of the three series was identical. For the sake of convenience the three series may be designated the deficiency series, the optimum series, and the toxicity series, respectively. In all cases the experimental period, during which the cultures were under treatment, was continued until pronounced visible symptoms of boron deficiency appeared in the plants of the deficiency series, and of boron toxicity in the plants of the toxicity series. This required different intervals of time for the different species. The cultures were conducted under a system of continuous flow of the culture solution at a rate sufficiently high to prevent material alteration in the proportions of nutrient ions through the activity of the plants.

At the end of the experimental interval the various tissues of the plants were analyzed for calcium and for boron and an attempt was made to differentiate between total and soluble or active quantities of the elements in the tissues tested, in the hope that such a procedure might throw some light upon the connection between these two elements in the metabolic activity of the plants. While the experimental evidence reveals absolutely no information concerning the actual physiological function of either boron or calcium in the plant, it does indicate at least one important rôle which the trace element boron plays in the functional processes of the major element calcium.

From a consideration of the experimental data (**7**) it became immediately obvious that the boron in the tissues of the particular species in question (corn) was virtually in a completely soluble state and could therefore be

assumed to be in a mobile, active condition. It was obvious also that its accumulation in the tissues was determined by its concentration in the nutrient substrate. When now the experimental data relating to boron were examined in connection with corresponding data representing the status of calcium in the plant, the outstanding feature which was at once apparent was the almost perfect correlation between the boron of the tissues and the soluble or active calcium of the tissues. On the other hand, there was no apparent relation between the boron content and total calcium in the tissues of this monocot, and there was no obvious relation between soluble and total calcium in tissues of these plants. It must be concluded, therefore, that the proportional part of the total calcium in the plant which was maintained in the soluble, active state in which it could be translocated from points of supply to centers of metabolic activity, was determined not by the total calcium content of the plant but by the supply of available boron in the corresponding tissues which in turn was determined by the boron concentration of the nutrient substrate. This peculiar property of boron by which calcium is rendered mobile and active is not confined to organic substrates. The principle involved is effectively utilized in certain industrial procedures to eliminate calcium in the soluble form from complex inorganic (**3**), as well as organic (**1, 4**) systems in which it occurs as an impurity during the process of large-scale preparation and manufacture of certain important commercial products.

The corresponding analytical data of a representative dicot, *Vicia faba*, were next considered. When these were treated in a manner similar to those of the monocot, corn, it was found that a relatively small fraction of the total boron in this dicot was soluble and in this respect the dicot was strikingly different from the monocot. But the soluble boron fraction of the dicot, like that of the monocot, was directly related to the total boron content of the tissues and also to the concentrations of this element in the nutrient substrate.

In the dicot, *Vicia faba*, the soluble calcium, as in the monocot, was directly related to and determined by the soluble boron fraction, which in turn was related to the total boron content and determined by the concentration of this element in the nutrient substrate. There was no direct relation between soluble and total calcium either in the monocot or in the dicot, but in the toxic range of boron concentrations of the substrate the accumulation of calcium was impeded, and this was directly associated with a corresponding retardation of the actual rates of calcium absorption and of the rates of growth.

One of the characteristic differences between the monocot and the dicot relative to this particular phase of inorganic nutrition was the fact that under identical experimental conditions with respect to the chemical and

physical properties of the nutrient substrate, the total calcium content and the total boron content were always much higher in the dicot than they were in the monocot. At present there is no adequate explanation for this fairly general phenomenon. In all of the species so far investigated the calcium and boron values for the dicot are usually much higher than those for the monocot. But this did not hold for the soluble fractions of these elements in the tissues of the plants. The soluble fraction of boron relative to the total was always much lower in the dicot than in the monocot and this invariably resulted in a low fraction of soluble calcium, relative to the total.

TABLE I

AVERAGE SOLUBLE BORON AND AVERAGE SOLUBLE CALCIUM, PERCENTAGE OF TOTAL, PRESENT IN THE TISSUES OF MONOCOTS AND DICOTS

RANGE OF SUPPLY	MONOCOTS		DICOTS	
	SOLUBLE BORON	SOLUBLE CALCIUM	SOLUBLE BORON	SOLUBLE CALCIUM
	%	%	%	%
Deficiency boron range	62.7	28.3	6.5	16.5
Optimum boron range	78.4	34.7	28.7	22.9
Toxicity boron range	86.1	55.7	32.2	25.4

This is clearly brought out in table I showing percentages dealing with relative solubilities of the two elements in representative species of the two groups of plants, monocots and dicots, so important in general agriculture. A consideration of these data provides an explanation for the well-known fact that the boron requirement of the monocot for normal growth and development is very much lower than is that of the dicot but equally essential for both, and it accounts also for the frequent appearance in the literature of statements to the effect that boron is not essential for growth and development of the agricultural monocots such as oats, wheat, and some grasses. The optimum boron requirements of the dicots thus far investigated were from five to ten times as high as those of the monocot, but this is only a rough estimate, since it is extremely difficult to evaluate accurately, in terms of growth criteria, the effective concentrations of boron in the extremely low ranges required by the monocots.

In the course of these investigations it was shown frequently that if at any stage in the cycle of these experimental plants the boron was excluded from the nutrient substrate so that a deficiency of active boron occurred within the plant, such deficiency rapidly destroyed the potential metabolic possibilities of the calcium even when calcium was present in adequate concentrations both in the tissues and in the substrate. Under such conditions

the plant quickly manifested deficiency symptoms. Were they boron deficiency symptoms or were they calcium deficiency symptoms? Who knows, since a deficiency of available boron in the tissues was directly associated with an inadequate supply of active calcium, and since the symptoms produced by an apparent deficiency of one element were identical with those produced by a deficiency of the other? If, on the other hand, boron was maintained in adequate concentration and calcium was excluded from the nutrient substrate at any stage in the growth cycle, calcium deficiency symptoms did not manifest themselves until the calcium previously acquired by the plant became inadequate in quantity to maintain the normal growth status of the plant. This required a relatively long period of time, since in the presence of an adequate supply of active boron the calcium already in the plant functioned quite effectively.

There is considerable experimental evidence to indicate that boron is a vital factor in the processes involved in organic syntheses. During the course of microchemical investigations of the terminal meristematic tissues of both monocots and dicots, it was discovered incidentally through the use of methods of staining, that striking differences occurred in both the pectin content and the fat content of the cells of the tissues from plants grown in the deficient, the optimum, and the toxic boron concentration ranges. An attempt was then made to determine qualitatively and roughly by quantitative methods whether the differences observed might in any way be related to the effective boron concentrations in the nutrient substrate and in the tissues. It is interesting and perhaps important that plants grown within the range of deficient boron concentration yielded strong positive tests for pectins and negative tests for fats. Plants grown within the range of toxic boron concentrations always yielded negative tests for pectins and strong positive tests for fats. Plants grown in the range of optimum boron concentrations always showed the presence of both pectins and fats. It must be strongly emphasized that these observations and tests are merely suggestive and will require confirmation by repeated checks and tests making use of much more exact methods of quantitative analyses than those employed in these preliminary investigations which strongly suggest that boron plays an important indirect rôle in carbohydrate synthesis and fat metabolism. In such a rôle its effects can not be direct but its influence is made effective through other factors in some such manner as has already been discussed, and these factors may be inorganic in nature, such as calcium involved in the mechanisms of organic synthesis. This becomes understandable in view of the fact that in green plants with high concentrations of active calcium, carbohydrate synthesis may give way to the formation of fats and fatty substances (**9**). Further experimental evidence also suggests that boron may play similar indirect rôles in certain metabolic processes in-

volving the cations and perhaps the anions of major elements other than calcium (**11**), but this evidence is too fragmentary and inadequate for broad generalization.

There is another important phase of trace element nutrition which involves the activity of the two elements iron and manganese. That these two elements are intimately interdependent in their effects upon the plant, and that the nature of the activities of one of these is determined by the proportionate presence of the other, can no longer be doubted. Such interrelationships between these two elements, relative to their active influence in the metabolic system of the plant, have been suggested by many investigators. These suggestions have usually been associated with the fact that symptoms of chlorosis, and other characteristic effects resulting from deficiency or excess of one of these elements, are either identical with or reciprocally related to each other.

The theoretical explanation of the rôle which manganese plays in the metabolic processes, in which iron also assumes an important rôle, revolves around two facts: first, that the active, functional iron in the tissues is in the reduced state, that is, in the ferrous condition; and, second, that the oxidizing potential of manganese is higher than that of iron. If iron in the ferric state is absorbed by the plant, much of it is immediately reduced to the ferrous form under the powerful reducing systems in the cell, unless it is restrained by some counterreactant. If such a reactant is not present, or is present only in deficient quantity, a very low concentration of iron in the active state may become a powerful toxic agent resulting in a type of chlorosis which is readily recognizable as an iron toxicity symptom, or it might with equal correctness be designated as a manganese deficiency symptom. If, however, a strong oxidizing agent with an oxidizing potential considerably higher than that of iron is present in adequate concentration, the reduction of iron is restrained, or if iron is already present in the reduced state, it may be oxidized to the ferric state, in which condition it may be precipitated, probably in the form of ferric organic complexes. Manganese is the element which possesses the chemical characteristics necessary for such a theoretical system, and there is little doubt that it reacts with iron in the plant in accordance with the chemical principles suggested and in some such manner as is here described, but the sequence of events and the details of the exact processes involved are utterly complex and not at all understood.

Assuming that such a dynamic relationship exists in the plant between iron and manganese, it then becomes evident that so long as a supply of iron and manganese is available in the nutrient substrate a dynamic equilibrium system involving both oxidized and reduced ions of both iron and manganese must be maintained. Theoretically, however, complete oxidation or complete reduction cannot be attained in such a system so long as a supply of

these elements is available in the nutrient substrate and absorbed by the plant; but the oxidation of ferrous ions to ferric ions and accordingly the precipitation of iron in the form of ferric organic complexes is determined by the relative quantity of manganic ions present in the system. Thus, when the relative proportion of manganic ions in the plant is high, the active ferrous ions are maintained at a proportionately low level through this process of oxidation reduction. But when the relative proportion of manganic ions is low, the active iron, which is capable of functioning in the metabolic processes, is maintained at a proportionately high level. When these active ferrous ions become excessive in the tissues through a shift in the dynamic equilibrium system in their direction because of a proportionately low supply of manganese in the nutrient substrate, the plant will manifest this excessive iron activity by the development of toxicity symptoms. On the other hand, when the equilibrium is shifted in the opposite direction, as it is with relatively high manganese in the substrate, so that a deficiency of active iron results, the plant will manifest this by the development of symptoms associated with iron deficiency. These symptoms might with equal correctness be designated as manganese toxicity symptoms.

These processes have been followed analytically both with plant materials and in inorganic systems and the several steps described have been experimentally verified. Their effects upon the plant can readily be demonstrated and this has been done in the following manner: Three series of cultures were grown in a standard culture solution. In each series of cultures the iron supply was maintained at a constant level. This was very low in one series, approximately optimal in the second, and high in the third. In each series the manganese concentration range extended from very low to very high concentrations so that in one direction the only variable factor was that of iron concentration and in the other direction the only variable factor was that of manganese concentration.

Inspection of the analytical data from these series of cultures brings out the fact that a shift from low to high iron in the substrate causes a corresponding shift in the concentration of manganese required to produce good growth of the plants, free from symptoms of toxicity or deficiency. In other words, when iron is low in the substrate good growth is obtained only when manganese is correspondingly low, and when iron is high in the substrate good growth is obtained only when manganese is correspondingly high. The data further show that good growth can be obtained with a relatively wide range of iron concentrations in the nutrient substrate but only when accompanied by a corresponding range in the concentrations of manganese. This demonstrates the importance of maintaining in the nutrient substrate the proper ratio of iron to manganese, which has a value of approximately two (2) for the species investigated although good growth may be obtained

within a limited range of values for this ratio. It demonstrates also, that, within certain limits not attained in these investigations, the total supply of these two elements is not at all important, provided only that the proper ratio of active concentrations of iron to manganese is maintained. The analytical data show that within the tissues of the plant a similar relationship exists, not between total quantities of iron and total quantities of manganese, but between active iron and active manganese, although here a somewhat greater fluctuation than that which is permissible in the nutrient substrate, may occur in the value of this ratio and still be consistent with good growth and development of plants which are free from pathological symptoms. The effective internal range of these ratio values extends from approximately 1.5 to 2.5 for the species investigated, but it is not expected that the same range of values would be effective with all species with reference to either the nutrient substrate or the active plant tissues.

As a final consideration and as the result of these studies, it may be pointed out that the rôles which have here been ascribed to the trace elements boron, iron, and manganese, important as they may be, are mere incidents in the whole complex maze of physiological functions which they may indirectly assume in the vital activities of any species. While it is at present impossible to assign any one particular process as the special function of a given trace element, it is probably safe to assume that each of these elements is a critical factor in every important physiological process involved in the nutrition of a plant.

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LITERATURE CITED

1. Anonymous. Calcium gluconate production speeded up. News Ed. Amer. Chem. Soc. **19**: 221. 1941.
2. ARNON, D. I. Micro-elements in culture-solution experiments with higher plants. Amer. Jour. Bot. **25**: 322–325. 1938.
3. BERGER, C. Borax in metallurgy and tanning. Rev. Gen. Chem. **16**: 133. Chem. Abs. **7**: 2373, Item 6.
4. CALVER, S. W. Comparison of the deliming action of hydrochloric, acetic, and boric acids. Jour. Int. Soc. Leather Trades Chem. **18**: 266–275. 1934.
5. JOHNSTON, E. S., and DORE, W. H. The influence of boron on the chemical composition and growth of the tomato. Plant Physiol. **4**: 31–62. 1929.
6. LIPMAN, C. B. Aspects of inorganic metabolism in plants. Ann. Rev. Biochem. **9**: 491–508. 1940.
7. MARSH, R. P., and SHIVE, J. W. Boron as a factor in the calcium metabolism of the corn plant. Soil Sci. **51**: 141–151. 1941.

8. PHILLIPS, W. R. The effect of boron on the respiratory behavior of tomatoes. Sci. Agr. **18**: 738–740. 1938.
9. PRIESTLY, J. H. The fundamental fat metabolism of the plant. New Phytol. **23**: 1–19. 1924.
10. STEINBERG, R. A. Rôle of molybdenum in the utilization of ammonium and nitrate nitrogen by *Aspergillus niger*. Jour. Agr. Res. **55**: 891–902. 1937.
11. WADLEIGH, C. H., and SHIVE, J. W. A micro-chemical study of the effects of boron deficiency in cotton seedlings. Soil Sci. **47**: 33–36. 1939.
12. WHITE, P. R. Does "C. P. grade" sucrose contain impurities significant for the nutrition of excised tomato roots. Plant Physiol. **15**: 349–354. 1940.
13. ————————. Plant tissue cultures. Biol. Rev. **16**: 34–48. 1941.

WATER BALANCE IN THE ONION ROOT: RELATION OF VOLUME INTAKE TO VOLUME EXUDATE OF EXCISED ROOTS AND ISOLATED ROOT SEGMENTS

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(WITH TWO FIGURES)

Introduction

In order to effectively study water balance and ion exchange in higher plants it is important to obtain quantitative data concerning the mechanism of transport in root tissue *per se*. Such knowledge is also of significance in cell dynamics since water balance is a property common to all cells. Although a careful study involving simultaneous determinaton of both absorption and exudation in a single isolated root or portion of root is essential to a complete analysis of the dynamics of polar transport in higher plants, no such study has appeared heretofore. The present investigation which was confined to excised onion roots and isolated root segments should be extended to include other types of roots including those which have been grown by tissue culture technique.

Young onion roots (less than two weeks old) grown in water culture show polar apical-basal differences in rates of water intake which are characteristic of root tissue since these differences are maintained both before and after the roots have been isolated from the bulb and developing leaves; although water intake occurs at all levels, higher rates appear in the relatively more basal regions (**16**).

Unit volume of transport at a given axial level in an excised onion root may be just as large or larger than when it had the supposedly added force of the bulb and developed leaves to reinforce or supplant its own force in the transport of water in a saturated atmosphere. There is, of course, no conclusive evidence which shows whether or not the bulb and developing leaves exert a force which supplants that of the root under the given conditions. Although in some roots the rates are lower immediately after severing the root from the shoot, fifteen hours later the rate of transport by the excised root is frequently greater than that during a previous corresponding intact period. It has been shown (**16**), however, that rates at given axial levels of young roots increase with time in both the intact and excised state.

Fluctuations in the characteristic gradients of water intake occur along the longitudinal axis of intact and excised onion roots. Spontaneous variations in rates, sometimes opposite in direction, are also simultaneously exhibited by contiguous levels from one two-hour interval to another (**15, 16**).

Water loss from epidermal cells has never been observed at any axial level in the healthy growing onion root; but previous experiments have not shown whether or not a decrease in rate was produced by liquid loss from minute cellular areas. The area at a single potometer contact in experiments on several hundred roots was seldom less than 2, nor greater than 4, square millimeters; if liquid loss occurred from minute cellular areas decreasing the total intake at a single potometer contact, such loss was obscured by an algebraic over-all intake at a given level during the interval. JENNY, OVERSTREET, and AYERS (9) have demonstrated that intake of ions by some roots is not a unidirectional process and that the same ion species may move into and out of the root at the same time. Other ion exchange studies have also been made; MAZIA (13) showed that ion exchange occurs in Elodea; MULLINS and BROOKS (14) and BROOKS (4) have demonstrated radioactive ion exchanges in single cells. Is water absorption a unidirectional process? Does water loss occur when rates of intake decrease and exudation pressure falls? Does water loss enter into the maintenance of the "rhizosphere" around roots? SIERP and BREWIG (18) and BREWIG (2) have described water loss in the apical region of *Vicia faba* simultaneous with absorption in basal regions.

The appearance of small irregular oscillations in the "bleeding" of cut stems with root systems attached was recorded by BARANETZKY in 1877 (1). In a recent article by HEYL (7) similar phenomena are mentioned. No measurements were made by either investigator to determine the possibilities of parallel variations in water intake and exudation. GROSSENBACHER (5) has described diurnal fluctuations in root pressure and volume exudation. Measurements of water intake to determine the presence or absence of parallel recurring maxima and minima in both absorption and exudation were not included in his investigations. In studies on the influence of the shoot on root permeability and resistance to water intake BREWIG used root systems with a portion of the stem attached (2). In a more recent article BREWIG (3) describes experiments with isolated root segments, but the object of the experiments differed greatly from those which are presented in this paper; BREWIG determined the effect of passing air over a portion of the root segment to simulate transpiration; he also used osmotically active solutions. In the present investigation no attempt was made to determine the effect of different agents on water transport. It appears to be the first study to include simultaneous measurements of absorption in contiguous regions and exudation in root tissue *per se* in a saturated atmosphere under constant external conditions. The technique used in the following experiments makes possible the simultaneous measurement of intake, outgo, and retention of water and therefore fulfills the fundamental experimental requirement in an adequate quantitative study of water balance.

Methods and results

Water transport in excised roots: relation of volume absorption to volume exudation

Calibrated potometers of small bore attached in a horizontal position to an upright glass rod within glass chambers provided a means of determining absorption rates and exudation in different root regions at the same time. Experiments were carried out in the dark with a saturated atmosphere inside the chambers. Room temperature did not vary more than $\pm 0.5°$ C. in the longest experiments. Condensed liquid which collected on the observation window was removed before each reading by careful manipulation of a "window wiper" from the outside. Further details of the apparatus may be obtained from previous publications (**15, 16**). Roots were obtained from onions (*Allium cepa*) grown in aerated nutrient solutions or in soil.

Simultaneous measurements of water intake and of exudation of over 100 individual excised roots reveal that fluctuations in both volume of inflow and outflow occur from one two-hour interval to another and that the variations in volume outgo may occur independently of variations in intake. The ratio of volume output (exudation) to volume input (absorption) during any two-hour interval may be equal to, slightly greater, or less, than 1. Characteristic results are represented by the data in table I; additional data would be repetitious.

The plants from which the data in table I were obtained were placed in the experimental chambers the night preceding the experiment; excisions were made the following morning without removing the chamber covers in order to eliminate any marked change in humidity within the chambers. Only a portion of the potential absorbing surface of each root was utilized since not more than four potometers were used for absorption; the absorbing area at a single potometer contact was not greater than 3.69 sq. mm. and not less than 1.45 sq. mm. The potometers were spaced 10 mm. apart with the bottom potometer at the apex. Readings were made at 2-hour intervals throughout a 24-hour period following an initial reading at 10 A.M. Elongation at decreased rates continued in the excised roots from 6 to 12 hours.

All four roots manifested marked oscillations of the ratio of volume inflow (absorption) to volume outflow (exudation) from interval to interval. Independent fluctuations in both input and output were apparent; frequently diminution of intake simultaneous with acceleration of outflow, or *vice versa*, was displayed.

The greatest range of variation of ratios appears in root II, which exhibited a ratio of 0.05 during the second and 1.05 during the tenth interval. All four roots manifested a ratio less than unity during the first interval. This is usually the case immediately following excision. Although precautions were taken to supply the plant with an abundance of water preceding

TABLE I

VOLUME OF TAP WATER ABSORBED FROM FOUR POTOMETERS AND SIMULTANEOUS EXUDATION OF FOUR SINGLE ROOTS DURING TWO-HOUR INTERVALS*

Root	Interval	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	Twelfth
	Time	10 A.M. to 12 P.M.	12 to 2 P.M.	2 to 4 P.M.	4 to 6 P.M.	6 to 8 P.M.	8 to 10 P.M.	10 to 12 P.M.	12 to 2 A.M.	2 to 4 A.M.	4 to 6 A.M.	6 to 8 A.M.	8 to 10 A.M.
I	Absorption ($mm.^3$)	1.330	1.055	1.119	1.638	1.557	2.139	2.173	2.432	2.273	2.276	2.027	2.171
	Exudation ($mm.^3$)	0.389	0.389	0.649	0.649	1.525	1.927	2.109	2.011	2.463	2.312	2.028	2.019
	Ratio $\frac{\text{Vol. Exud.}}{\text{Vol. Abs.}}$	0.29	0.37	0.58	0.39	0.98	0.90	0.97	0.83	1.08	1.01	1.00	0.93
II	Absorption ($mm.^3$)	0.733	1.207	0.604	1.173	1.386	1.974	1.828	1.907	2.429	2.368	2.333	2.363
	Exudation ($mm.^3$)	0.098	0.065	0.293	0.587	0.978	2.043	1.900	1.830	2.210	2.501	2.340	2.103
	Ratio $\frac{\text{Vol. Exud.}}{\text{Vol. Abs.}}$	0.13	0.05	0.48	0.50	0.71	1.03	1.03	0.96	0.91	1.05	1.00	0.89
III	Absorption ($mm.^3$)	1.315	1.200	1.378	1.381	1.135	0.746	0.779	0.617	0.975	0.986	0.908	0.828
	Exudation ($mm.^3$)	1.233	1.200	1.427	1.492	0.892	0.568	0.709	0.635	0.917	0.789	0.744	0.649
	Ratio $\frac{\text{Vol. Exud.}}{\text{Vol. Abs.}}$	0.94	1.00	1.04	1.08	0.79	0.76	0.92	1.03	0.94	0.80	0.82	0.78
IV	Absorption ($mm.^3$)	0.211	0.293	0.552	0.601	0.762	1.006	1.830	2.223	1.931	1.391	0.842	0.763
	Exudation ($mm.^3$)	0.195	0.260	0.373	0.503	0.616	0.905	1.590	2.178	1.924	1.441	0.681	0.600
	Ratio $\frac{\text{Vol. Exud.}}{\text{Vol. Abs.}}$	0.92	0.89	0.68	0.84	0.81	0.90	0.87	0.98	1.00	1.03	0.81	0.79

* Roots I, II, III, and IV were 5 days old and 44.8, 49.9, 52.3, and 51.1 mm. in length, respectively, when initial readings were made at 10 A.M.

excision, it may be that the bulb with developing leaves produced a water deficit in the root tissue. Water deficits and the requirements of growth might account for lower ratios during the first half of the 24-hour period before growth had ceased and before a ratio of 1.0 was attained. Root III, however, exhibited equal outflow and inflow during the second, third, and fourth intervals when growth took place and saturation deficits may have been present. Root III is also an exception to the fact that in most cases there is a marked increase in the magnitude of both inflow and outflow with time. Many young roots manifested a sixfold to tenfold or greater volume increase with time during the 24-hour experimental period. Exudation usually reached a maximum before the 24-hour experimental period ended. Whether or not this behavior indicates a diurnal cycle has not as yet been determined. During consecutive two-hour intervals a twofold and even a threefold change in volume exudation was sometimes observed.

Absorption and exudation of relatively apical segments

It was early observed that if an excised root was converted into an isolated segment by removal of the root cap region with a clean cut excision, exudation occurred solely at the basal end of the segment whether or not it was in an horizontal or a vertical position. When an excised root 65 mm. in length was cut in two, exudation appeared at both ends of the upper half. Individual experiments were made on 35 roots to determine the exact level at which exudation appeared at both apical and basal ends of an intermediate segment.

Measurements of simultaneous absorption and exudation were made on the intermediate segments which were isolated by removing different lengths of the apex and base of excised roots. The lengths of the tip removed differed by increments of 0.5 to 16 mm. Five potometer tubes were used; the cut ends of the intermediate segments extended into tubes 1 and 5, and water was absorbed from tubes 2, 3, and 4 placed between them. The potometers were 10 mm. apart. Owing to space limitations, data from only 9 roots are given in table II.

When less than 2 mm. was removed from the apical end of the excised root, the intermediate segment manifested elongation. This was due to the fact that the greatest amount of elongation in onion roots occurs in the second millimeter from the apex which in this case had not been removed. At the beginning of the experiment a small drop of water was placed over each cut end in potometers 1 and 5; this was usually absorbed by the apical end as shown by segments A, B, and C in roots I, II, and III (table II), which exhibited no exudation at the apical end. Segments with 5 to 16 mm. of the tip removed displayed absorption or exudation at the apical end during alternate intervals. The magnitude of total absorption, however, exceeded

TABLE II

SIMULTANEOUS VOLUME ABSORPTION AND VOLUME EXUDATION OF ISOLATED SEGMENTS MANIFESTING LITTLE OR NO EXUDATION AT THE APICAL END*

Root	Age of root	Total length of root	Length of basal segment removed	Length of apical segment removed	Length and designation of intermediate segment in potometers	Time	Length of interval	Potom. 1 Volume exuded at apical end	Potom. 1 Volume absorbed by apical end	Potom. 2 Volume absorbed	Potom. 3 Volume absorbed	Potom. 4 Volume absorbed	Potom. 5 Volume exuded at basal end	Ratio: Volume exuded / Volume absorbed
	days	*mm.*	*mm.*	*mm.*	*mm.*		*hr.*	*mm.*^{3}	*mm.*^{3}	*mm.*^{3}	*mm.*^{3}	*mm.*^{3}	*mm.*^{3}	
I	5	60.8	17	0.5	43.3 A	8 A.M. to 6:30 P.M.	10.5	0.0	0.093	0.648	0.277	0.740	1.370	0.71
						6:30 P.M. to 9 A.M.	14.5	0.0	0.0	2.830	1.295	3.328	2.330	0.98
II	5	63.0	18	2.0	43.0 B	8:05 A.M. to 6:35 P.M.	10.5	0.0	0.424	0.505	0.685	1.302	2.494	0.85
						6:35 P.M. to 9:05 A.M.	14.5	0.0	0.0	1.873	1.597	2.084	5.282	0.95
III	6	52.7	6	3.5	43.2 C	10:30 P.M. to 8 A.M.	9.5	0.0	0.091	0.603	0.603	1.043	1.972	0.84
						8 A.M. to 2 P.M.	6.0	0.0	0.617	0.522	0.782	0.929	2.852	1.00
						2 P.M. to 8 P.M.	6.0	.....	0.782	1.043	1.108	1.304	4.322	1.02
IV	6	57.5	9	5.5	43.0 D	10:35 P.M. to 8 A.M.	9.5	0.124	0.0	0.630	0.456	0.815	1.114	0.65
						8 A.M. to 2:05 P.M.	6.0	0.0	0.685	0.300	0.489	0.980	2.208	0.90
						2:05 P.M. to 8 P.M.	6.0	.....	.....	0.326	0.489	0.815	1.543	0.95
V	7	70.1	20	7.5	42.6 E	8 A.M. to 1 P.M.	5.0	0.0	0.033	0.408	0.493	0.561	1.536	1.03
						1 P.M. to 6 P.M.	5.0	0.072	0.0	0.245	0.371	0.897	1.463	1.01
VI	5	69.1	16	10.0	43.1 F	8 A.M. to 4 P.M.	8.0	0.628	0.0	0.333	0.518	0.831	0.777	0.83
						4 P.M. to 8 P.M.	4.0	0.167	0.0	0.285	0.728	1.730	1.877	0.99
						8 P.M. to 10 A.M.	14.0	.....	0.178	0.851	1.300	1.921	4.440	1.04
VII	5	64.7	9	12.5	43.2 G	3 P.M. to 8 P.M.	5.0	0.0	0.163	0.424	0.783	1.415	2.680	0.96
						8 P.M. to 8 A.M.	12.0	0.0	0.310	1.482	2.235	3.226	8.413	1.02
VIII	5	62.2	5	14.0	43.2 H	8:05 A.M. to 4:05 P.M.	8.0	0.098	0.0	0.627	0.764	0.894	1.821	0.83
						4:05 P.M. to 8:05 P.M.	4.0	0.0	0.390	0.185	0.813	0.975	2.398	1.04
						8:05 P.M. to 10:05 A.M.	14.0	.....	0.169	0.715	2.760	2.760	6.328	1.00
IX	5	64.1	5	16.0	43.1 I	8:10 A.M. to 4:10 P.M.	8.0	0.146	0.0	0.540	0.390	0.683	1.434	0.98
						4:10 P.M. to 8:10 P.M.	4.0	0.0	0.065	0.390	0.358	0.943	1.450	0.84
						8:10 P.M. to 10:10 A.M.	14.0	.....	1.010	1.430	1.50	2.940	6.850	0.98

* Experiments were carried out at 25° to 27° C.

that of total exudation in all but segments E and F from root V and VI. When more than 16 mm. was removed in roots of this age exudation usually exceeded absorption at the cut apical end.

The transport ratio of volume outflow to volume inflow $\frac{\text{total exudation}}{\text{total absorption}}$ was seldom unity during the first interval following excision. With time the ratio approached or reached unity, the isolated segment transporting all the water which was simultaneously absorbed; a ratio of one was not reached by growing segments. A comparison of the ratios during equal intervals of time and constant temperature as in segments C, D, and E from roots III, IV, and V in table II show that exudation may be equal to, slightly greater, or less than, absorption during consecutive intervals. In most cases in which the isolated segments were placed in the chambers the night before, the segments manifested oscillating ratios during two-hour intervals the following day but a ratio of unity when total outflow and inflow (sum of intervals) was compared. In table II, segments F, G, and H show ratios of one during the 12- or 14-hour period of the second or third interval; in this case, however, it is not known if the ratios were maintained from instant to instant.

The average rates of water intake from potometers 2, 3, and 4 were determined but are not given in table II owing to space limitations. The highest rates appeared at the relatively more basal levels and fluctuations of rates occurred changing the gradient of distribution from time to time. Comparisons of rates of water absorption of a given length of root when the root was intact (saturated atmosphere), when it had been excised, and finally when completely isolated from the root, showed that the given root tissue manifested the same type of behavior it exhibited before it was cut from the excised or intact root.

Direction of transport in isolated contiguous segments

Figure 1 is a diagrammatic representation of total outflow and of average rates of intake of water in three segments cut from a 78-mm. root 5 days old. The segments were placed in a vertical position with 5 horizontal potometers attached, two (designated by arrows 1 and 5, fig. 1) to collect exudate and three (designated by arrows 2, 3, and 4, fig. 1) filled with tap water. The highest average rate of intake appeared in the middle segment at a level 45 mm. from the apex. Comparison of apical and basal outflow in each segment showed that the direction of outflow in the apical third was entirely basal against gravity; in the middle and basal thirds outflow occurred in two directions; in both segments the basal outflow was greater. The total outflow in the apical segment was relatively low, but in this case not only were the rates of intake also lower but growth of the segment had taken place. Although the highest rate of intake occurred in the middle third, greater

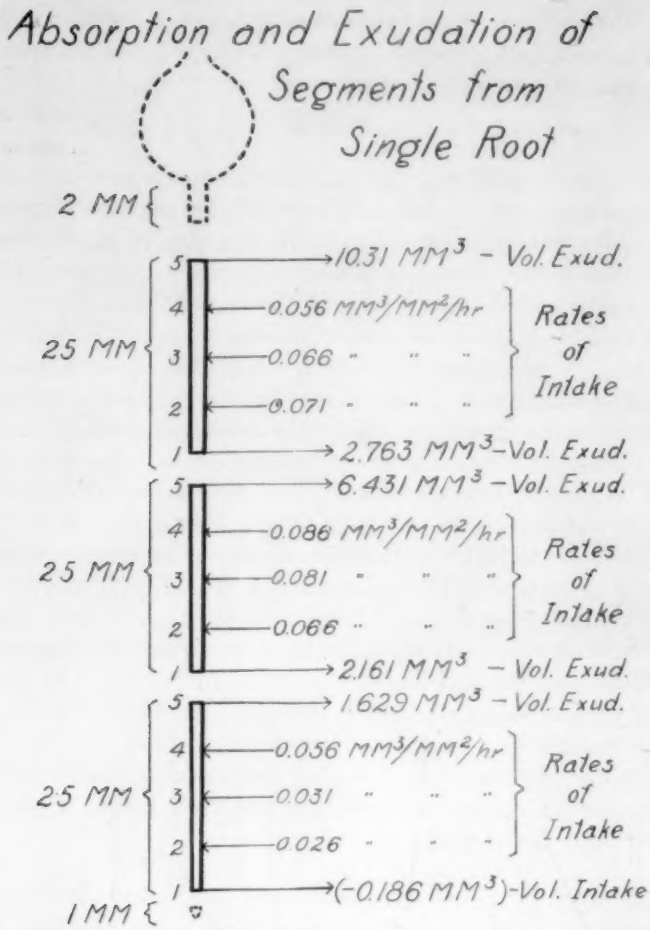

FIG. 1. Diagram of total volume outflow and average rates of intake of water in isolated contiguous segments of the same root. Positions of the potometers are designated by the arrows which are numbered 1, 2, 3, 4, and 5. Observations were made during a 20-hour period.

quantities of water were absorbed and transported by the basal third which was greater in diameter and, therefore, the area of absorption at the 3 potometers was correspondingly greater. The results obtained from this root are typical of segments of the same length and relative position cut from roots 75 to 100 mm. in length. Occasionally a basal segment exhibited greater exudation at the apical end of the segment but outflow occurred in both directions. When, however, basal segments were isolated from older and longer roots (over 200 mm. in length) and placed in their normal upright position with respect to gravity the direction of transport in the upright position was toward the apical end.

Figure 2 shows volume outflow of longer segments from two older roots designated as A and B. Observations were made during a 15-hour period with tap water in three potometers placed between those into which exudation flowed. In both roots, the relatively more basal segments manifested exudation at the apical end only. As indicated by the negative (–) sign most of the water covering the cut basal end disappeared. Since the vessels were open at both ends, this disappearance was caused by gravity, the water appearing in the potometer at the apical end in each case. In root A, no

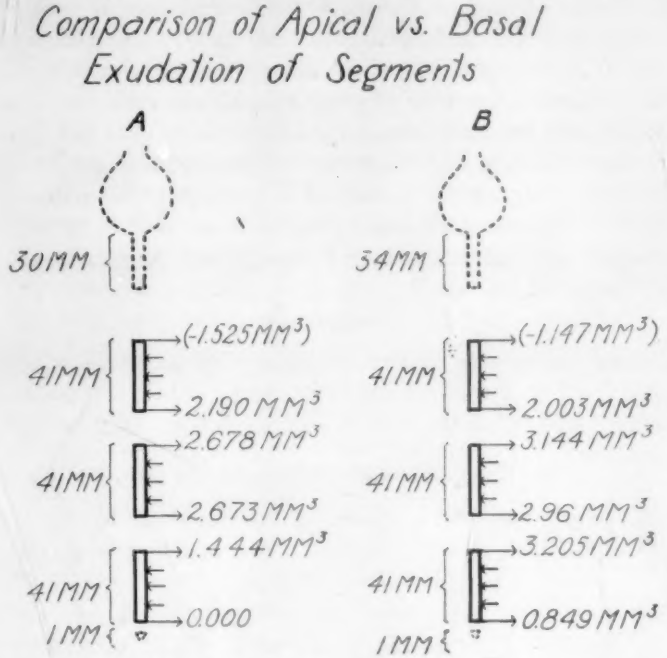

FIG. 2. Comparison of volume exudation at the ends of isolated contiguous segments of two roots designated as A and B. Positions of potometers designated by arrows.

exudation appeared at the apical end of the apical segment; root B, on the other hand, manifested a small quantity of exudation at this end. The second segment from the apex manifested exudation at both cut ends; the volume flow in each direction was equal in root A and practically so in root B.

The direction of outflow exhibited by the segments of the above two roots is characteristic of segments cut from older and longer roots. When basal segments were cut from roots 300 to 400 mm. in length and a drop of water was placed over each end, with the segment in the upright position, the water from the basal end flowed down the vessels appearing in the bottom (apical)

potometer immediately after; placed in an inverted position, the reverse was true; placed in a horizontal position equal amounts of exudation appeared in potometers at the two ends of the segments when water was absorbed from potometers in between.

The direction of transport through the stele of an isolated segment depends upon its age and upon the level of the segment relative to its position on the longitudinal axis. Segments from the apical third of young roots less than 65 mm. in length and less than a week old exhibit basal outflow only in an upright, inverted, or a horizontal position; segments from the middle and basal third exhibit outflow at both ends in all three positions. In isolated pieces of roots of this length the direction of transport is chiefly basal, structural features apparently playing a dominant rôle. If, however, segments are removed from levels near the bulb in roots over 200 mm. in length and 3 or more weeks old, the direction of transport depends upon the position of the isolated segment with respect to gravity. This indicates that the vector forces of transport are chiefly radial in isolated segments which are relatively older whereas in younger segments both longitudinal and radial factors are involved.

Discussion

It is important to note that the present investigation is concerned with *volume transport* and not pressures as such. Since external conditions were carefully controlled, the factors which brought about variations in the ratio of $\frac{\text{outflow}}{\text{inflow}}$ had their origin in changes in the root itself. That these factors are sharply localized is shown by the simultaneous changes in rates at the different levels of water intake. The total intake during any one interval is a summation of the local changes. Whether cells gain or loose water from one another is not shown but evidently the factors involved alter the flux relations of water in individual cells. This indicates the unequal distribution of *forces* (SHULL, **17**) throughout the isolated root tissue under the given conditions. The axial gradient of intake at any one moment is the algebraic sum of individual cellular activities; it varies in relation to both time and space under the particular conditions employed. The data furnish no evidence to indicate whether or not the opposite variations of outflow and inflow are produced by separate mechanisms. It is not believed that they are produced by faulty technique; similar experiments, however, should be carried out by other investigators to establish this property of isolated roots.

With respect to water balance, determination of output, especially during short intervals, is not necessarily a measure of input and *vice versa*. This may be true in all types of roots; if so, conclusions regarding water absorption are not valid when based upon measurements of exudation and

not water intake itself. SPEIDEL (**19**) made parallel determinations of water intake and exudation in the decapitated root system of Plectranthus. He found that volume intake was greater than volume outflow throughout a 24-hour period. Similar results have been obtained with onion roots in a few experiments. In most cases, however, where intake was greater than outflow immediately after excision a ratio of unity was reached before 24 hours had passed.

The magnitude of both volume inflow and outflow increased with time after excision. It may be that the change in rates with time indicated an obscure periodicity. BARANETZKY (**1**) observed two-hour oscillations in the "bleeding" of decapitated root systems in *Ricinis insignia* plants 2 weeks old with an absence of decided periodicity at this age; but when observations were made on plants 5 weeks old, pronounced periodicity was noted.

Examination of GROSSENBACHER's curves (**6**) obtained from topped Helianthus plants shows two- and threefold variations of rates of outflow between maxima and minima of 24-hour cycles under constant external conditions. Throughout a 12-hour period isolated onion roots frequently manifest a sixfold increase in rate of outflow. KRAMER (**11**) attached the cut stems of exuding cotton stem-root systems to a vacuum pump and noted (p. 486) that "four or five times as much water exuded under the reduced pressure as had exuded during the same period of time due to 'root pressure' alone." This change is no greater than that observed in a similar 12-hour period with onion root tissue in the absence of applied suction; in the onion root the change occurred under constant external conditions and was a property of the tissue itself; whether or not a similar capacity is present in cotton roots has not been shown. By attaching topped tomato root systems to a vacuum pump and lowering the pressure KRAMER (**12**, p. 787) "approximately doubled" the rate of exudation. Again it is interesting to note that single excised onion roots under constant conditions will do the same without artificially applied suction during consecutive two-hour intervals. KÖHNLEIN (**10**) and others have also applied suction to topped plants and obtained an increase in exudation; it may be that a similar increase in magnitude would have been manifested by the excised root tissue over a period of time under constant external conditions without the application of the suction pump. BREWIG (**3**), however, calls attention to the gradual decline of rate of exudation following excision of roots of *Vicia faba;* he maintains that in his experiments the rôle of "bleeding" can be ascertained only if measured immediately after cutting. When SPEIDEL made parallel measurements of water inflow and outflow in the decapitated root systems of Plectranthus he obtained a sharp increase in both, followed by a progressive decrease with time during a 24-hour period (**19**, fig. 23, p. 102). SPEIDEL attributes the steep descent of both outflow and inflow and the absence of

periodicity to oxygen deficiency produced by a gradual decrease of oxygen in the closed potometers. Closed potometers were not used in the present investigation.

JAMES and BAKER (**8**) described experiments with cut pieces of Sycamore roots which manifested uptake of water by the morphologically lower end and exudation at the upper end. They maintain that "the exudation is always in the morphologically upward direction even when the vessels have been opened at both ends, and will allow water to pass freely in the longitudinal direction. A drop of water placed at the physically upper end of the piece of root immediately causes a drop to appear at the lower end." Similar phenomena were not observed in pieces of onion root which manifested a longitudinal flow of water down the vessels when a drop of water was placed at the upper end of the root in a vertical position. Such pieces manifest exudation at either the distal or proximal end depending upon whether or not the segment is in an upright or inverted position; when oriented horizontally, exudation appears at both ends. JAMES and BAKER believed that their experiments furnished evidence that exudation from vessels did not take place. Although exudation from vertically placed basal segments cut from relatively old roots appeared in the lowest of a series of horizontally arranged potometers, the present experiments on onion roots do not furnish evidence for or against the possibility of phloem exudation since exudation from the phloem at the upper end of the segment would flow down the open vessels into the lowest potometer. The experiments do show, however, that epidermal absorption takes place in the absence of vessels filled with osmotically active solutes; forces outside of the vessels play a dominant rôle in the mechanism of intake in this case.

BREWIG (**2, 3**) maintains that variations of intake observed in the different root regions of *Vicia faba* are dependent upon transpiration. As mentioned earlier he passed air over portions of isolated roots and root segments to simulate transpiration. The experiments on the onion root show that fluctuations of intake in isolated roots occur in a saturated atmosphere under constant external conditions; in this case the regulatory phenomena are not associated with transpiration or evaporation from one end of the segment.

HEYL (**7**) has recently reviewed various theories dealing with the phenomena of "bleeding." He discusses theories which deal with tissue potentials, electro-endosmotic flow, varying conditions of the plasma membrane, unequal osmotic forces at opposite sides of the cell, variations in the distribution of osmotically active substances in the cell wall, rhythmic pulsation processes in living cells, variations in the osmotic suction strength of the vessel liquid, expansion and contraction of the cell or vessel walls, local differences in the osmotic pressure at different regions of a tissue, and changes

in pressure in the vessels as a result of injury. Whether or not one or more of the theories actually does apply to onion root tissue *per se* is only conjecture at the present stage of the work.

Summary

1. Simultaneous determination of water absorption and exudation of excised roots and pieces of roots revealed that fluctuations in both inflow and outflow occurred from one two-hour interval to another in a saturated atmosphere at a temperature which did not vary more than $\pm$ 0.5° C. during long intervals. The variations in outflow and inflow may be opposite in direction during any one interval.

2. The transport ratio of outflow to inflow, $\frac{\text{volume exudation}}{\text{volume absorption}}$, was seldom unity during the first interval following excision; with time the ratio approached or reached unity. Rates of both inflow and outflow usually increase with time during a 24-hour period following excision.

3. The direction of longitudinal transport through an isolated piece of root depends upon its age and upon its position on the longitudinal axis before cutting. Segments consisting of the apical third of young roots less than 70 mm. in length and less than a week old exhibit basal outflow only in an upright, inverted, or horizontal position with respect to gravity; segments from the middle and basal third exhibit outflow at both ends in all three positions. The direction of transport of basal segments cut from roots over 200 mm. in length and several weeks old depends upon the orientation of the segment with respect to gravity.

4. Isolated segments of the root manifest the longitudinal gradient of distribution of rates of absorption characteristic of the excised and intact state.

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LITERATURE CITED

1. BARANETZKY, J. Untersuchungen über die Periodizität des Blutens der krautartigen Pflanzen und deren Ursachen. Abh. d. Naturforsh. Ges. Halle **13**: 1–64. 1877.
2. BREWIG, A. Die Regulationserscheinungen bei der Wasseraufnahme und die Wasserleitsgeschwindigkeit in *Vicia faba* Wurzeln. Jahrb. wiss. Bot. **82**: 803–828. 1936.
3. ————. Auslösung leichter Wasserdurchlässigkeit an Wurzeln von *Vicia faba*. Planta **29**: 341–360. 1939.
4. BROOKS, S. C. Ion exchanges in accumulation and loss of certain ions

by the living protoplasm of Nitella. Jour. Cell. Comp. Physiol. **14**: 383–401. 1939.

5. GROSSENBACHER, K. A. Diurnal fluctuation in root pressure. Plant Physiol. **13**: 669–673. 1938.
6. ————. Autonomic cycle of rate of exudation of plants. Amer. Jour. Bot. **26**: 107–109. 1939.
7. HEYL, J. G. Der Einfluss von Aussenfaktoren auf das Bluten der Pflanzen. Planta **20**: 294–353. 1933.
8. JAMES, W. O., AND BAKER, H. Sap pressures and movements of sap. New Phytol. **32**: 317–343. 1933.
9. JENNY, H., OVERSTREET, R., AND AYERS, A. D. Contact depletion of barley roots as revealed by radioactive indicators. Soil Sci. **48**: 9–24. 1939.
10. KÖHNLEIN, E. Untersuchungen über die Höhe des Wurzel-Widerstandes und die Bedeutung aktiver Wurzeltätigkeit für die Wasserversorgung der Pflanzen. Planta **10**: 381–423. 1930.
11. KRAMER, P. J. The intake of water through dead root systems and its relation to the problem of absorption by transpiring plants. Amer. Jour. Bot. **20**: 481–492. 1933.
12. ————. The forces concerned in the intake of water by transpiring plants. Amer. Jour. Bot. **26**: 784–791. 1939.
13. MAZIA, D. The binding of Ca, Sr, and Ba by *Elodea* protoplasm. Jour. Cell. Comp. Physiol. **11**: 193–203. 1938.
14. MULLINS, L. J., AND BROOKS, S. C. Radioactive ion exchanges in living protoplasm. Science n. s. **90**: 256. 1939.
15. ROSENE, H. F. Distribution of the velocities of absorption of water in the onion root. Plant Physiol. **12**: 1–19. 1937.
16. ————. Comparison of rates of water intake in contiguous regions of intact and isolated roots. Plant Physiol. **16**: 19–38. 1941.
17. SHULL, C. A. Imbibition in relation to absorption and transportation of water in plants. Ecology **5**: 230–240. 1924.
18. SIERP, H., AND BREWIG, A. Quantitative Untersuchungen über die Wasserabsorptionszone der Wurzeln. Jahrb. wiss. Bot. **82**: 99–122. 1935.
19. SPEIDEL, BERTHOLD. Untersuchungen zur Physiologie des Blutens bei höheren Pflanzen. Planta **30**: 67–112. 1939.

AGENCIES AFFECTING THE PRODUCTION OF SUBSTANCE B BY *RHIZOPUS SUINUS*

CLAIR L. WORLEY

(WITH TWO FIGURES)

Introduction

In view of the wide distribution of growth factors[1] for microorganisms, it is not surprising to find that they can be obtained from other species of fungi and from bacteria, as well as from higher organisms. For many species there is no need to supply the factors, because they have the ability to synthesize these substances rapidly enough for optimal growth. Other species, although capable of manufacturing their own growth factors, do so at a suboptimal rate. In such cases, the deficiency of these substances becomes a limiting factor and their addition to the nutrient solution results in a marked acceleration of growth. Again, an organism may be totally unable to synthesize the needed compounds, and its growth, therefore, is strictly dependent upon the presence of these substances in the substratum. The amount of precise work that has been done on the classification of microorganisms into these three categories has been relatively large during the last decade. On the other hand, the quantity of work in relation to the agencies affecting the production of a growth factor, including vitamins, by the species of the first type mentioned, is very meagre and usually incidental to a larger problem.

NIELSEN (**5**) investigated the effect of the nitrogen source on the production of heteroauxin by *Rhizopus suinus*. Similar, though broader, tests were performed by BONNER (**1**) and by THIMANN (**8**). BOYSEN JENSEN (**2**) tested the effects of substrate, of temperature, and of pH on the heteroauxin production by *Aspergillus niger*. These constitute the known published papers that give consideration to physiological agencies affecting the production of growth factors by fungi. They are limited not only in number, but also in scope, and are concerned with the production of substance A (*i.e.*, heteroauxin). In no case have similar investigations been published relating to the production of substance B (an unknown substance increasing the dry weight production of *Aspergillus niger* and other species of fungi).

Procedures and methods

SPECIES EMPLOYED

The importance of the case history of all species of fungi used in physiological work has been mentioned frequently in the literature. It is now a

[1] "Growth factors" is used synonymously with growth substances, plant hormones, plant vitamins, growth accessory substances, etc.

well recognized fact that two fungi, isolated from the same culture, which show the same morphological and reproductive habits and are therefore classified as the same species, may respond in entirely different manners, physiologically. This physiological difference explains many of the discrepancies reported by various investigators working with the same taxonomic species.

A culture of *Rhizopus suinus* Niel. was obtained from stock cultures of the Botany Department of the University of Wisconsin. In preliminary tests, the fungus was cultured on NIELSEN's nutrient solution and under the conditions set forth by him. The filtrate was tested by the standardized pea, oat, and tomato-petiole methods for substance A, and by the *Aspergillus niger* method for substance B. In every case, positive qualitative results were obtained, and it was therefore concluded that this strain showed a physiological similarity to those employed by other workers. A transplant of *Aspergillus niger* was made from a stock culture maintained in the same Department. Preliminary tests showed that this strain was augmented by the Rhizopus filtrate but not by the Rhizopus cultural nutrient (see later). The strain of *Colletotrichum circinans* (Berk.) Vogl. was the one employed by WORLEY and DUGGAR (**11**) in an earlier investigation.

GENERAL PROCEDURES

The importance of details of technique in experimental work dealing with the growth factors of microorganisms has been emphasized in much of the literature. These are critically discussed in the reviews of PESKETT (**7**) and of KOSER and SAUNDERS (**4**). The main points listed in their reviews were considered in the conduction of the following series of tests. When dealing with the synthesis of an unknown substance by a new organism, however, the determination and the standardization of cultural techniques becomes an integral part of the investigations. Therefore, certain aspects of these cultural techniques are considered, one at a time, in the following investigations.

Throughout the entire investigation Pyrex glass containers were used. All apparatus was washed according to accepted methods and rinsed several times with distilled water, prior to use. The chemicals used were Baker's pure crystals, unless stated otherwise. And all cultures used as stocks for inoculation purposes were grown on solution III (see later). These stocks were cultured on slants in medium-sized test tubes, from which spores were washed with 20 ml. of physiological salt solution. The inoculum consisted of a 1-ml. aliquot of this preparation.

The standardized *A. niger* test method used is as follows. A 25-ml. portion of solution II and 5 ml. of the test solution were placed in each 125-ml. Erlenmeyer flask, in the method referred to as the 25 : 5 technique. In the 49 : 1 technique, 49 ml. and 1 ml. of the respective solutions were used. The

flasks containing the media were stoppered with cotton wadding and autoclaved for 15 minutes at 15 lb. pressure. After cooling, they were inoculated with an *A. niger* spore suspension according to the procedure already given. The inoculated cultures were grown at 31° ± 0.5° C., in diffused light, for 66 hours. This temperature was chosen because it proved to be the optimal temperature, as determined by preliminary tests. Since there is an increase in dry weight yield of *A. niger* until the 60th hour, and since there is a decline in the same caused by autolysis, after the 72nd hour, the mid-point, that is 66 hours, was chosen as the sampling time. At the termination of this time period the mats were filtered from the liquid, washed with distilled water, dried for three days in an electric oven at 92° to 96° C., cooled in a desiccator, and weighed.

The standardized *Rhizopus suinus* cultural method employed follows. Aliquots of 250 ml. of solution I were placed in liter Erlenmeyer flasks, stoppered with cotton, and autoclaved for 20 min. at 15 lb. pressure. After cooling, they were inoculated with a *R. suinus* spore suspension according to the given procedure. These cultures were grown at 25° ± 1° C., in diffused light, for 5 days. At the end of the cultural period the mats were separated from their substrata by filtration; the filtrates were autoclaved and stored for the Aspergillus tests, while the mats were washed, oven dried, cooled, and weighed. This general method was the basic cultural technique from which variations were made. In each test, one influencing agent is varied, and this variance is given with each test.

Nutrient solutions

The following nutrient solutions were used throughout this series of tests:

Solution I (for Rhizopus cultures)

NH_4-tartrate	10.228 gm. (M/18)
$MgSO_4 \cdot 7H_2O$	0.493 gm. (M/500)
KH_2PO_4	0.545 gm. (M/250)
Dextrose	10.005 gm. (M/18)
Ferric tartrate (sat. soln.)	3.000 drops
Dist. H_2O to 1000 ml.	

Solution II (for Aspergillus cultures)

NH_4NO_3	5.003 gm. (M/16)
$MgSO_4 \cdot 7H_2O$	0.616 gm. (M/400)
KH_2PO_4	0.619 gm. (M/220)
Sucrose	62.215 gm. (M/5.5)
Ferric tartrate (sat. soln.)	3.000 drops
Dist. H_2O to 1000 ml.	

SOLUTION III (FOR SLANTS AND FOR COLLETOTRICHUM PLATES)

NH_4NO_3	5.003 gm. (M/16)
$MgSO_4 \cdot 7H_2O$	0.616 gm. (M/400)
KH_2PO_4	0.619 gm. (M/220)
Dextrose	20.010 gm. (M/9)
Ferric tartrate (sat. soln.)	3.000 drops
Difco agar	17.000 gm.
Dist. H_2O to 1000 ml.	

STATISTICAL ANALYSES

During this investigation, which extended over a period of two and one-half years, the productivity of the strain of *R. suinus* slowly and progressively decreased. Thus, while the figures obtained for any one specific test are strictly comparable, this is not necessarily true for the figures of different tests. It seemed inadvisable, therefore, to average replicas and take their probable errors, as followed by the majority of workers in related fields. In lieu of the heretofore accepted procedure the following statistical method was employed. All measurements made, regardless of type, were recorded for the replicas of both controls and tests. Ratios, that is the test value divided by the control value, were calculated for every possible combination. The total number of ratios possible is the product of the number of test values and the number of control values. These ratios were averaged to obtain the value of the statistical factor (F) and its standard error (SE) was determined. This method of assay has two distinct advantages over the methods in common usage: (1) it readily permits the comparison of two different sets of data; and (2) the standard error obtained is greatly enlarged by extremes in test and control values and therefore is of greater significance. This is especially true when its value is relatively small.

Results

I. THE INFLUENCE OF THE WEIGHT OF THE RHIZOPUS MAT ON THE PRODUCTION OF SUBSTANCE B

The Rhizopus was cultured for five days in 250 ml. of solution I placed in 1-liter flasks. These were held at a temperature of 23° ± 1° C., while all other runs were cultured at 25° ± 1° C. The former were grown in the presence of a bottom light source obtained from six 100-watt Mazdas, placed at a distance of 16 inches and separated from the flasks by a glass plate and a one-inch running-water filter. Four filtrates of the Rhizopus cultures were tested by the Aspergillus method at three concentrations.

The results in table I fail to show any correlation between the amount of the growth factor produced and the amount of Rhizopus mycelium con-

TABLE I

Rhizopus suinus: THE RELATION OF THE DRY WEIGHT OF THE MYCELIUM TO THE PRODUCTION OF SUBSTANCE B (3 REPLICAS)

CULTURE	RHIZOPUS: DRY WEIGHT OF MYCELIUM	CONCENTRATIONS TESTED TS*: NUTRIENT: H_2O	ASPERGILLUS: DRY WEIGHT OF MYCELIUM (AVERAGE)	F ± SE
	mg.		*mg.*	
Control	.....	0: 40: 10	35.8	1.00
Test filtrate I	111.0	1: 40: 9	64.1	1.79 ± 0.024
		5: 40: 5	67.9	1.90 ± 0.014
		10: 40: 0	97.0	2.71 ± 0.022
Test filtrate II	108.0	1: 40: 9	42.6	1.19 ± 0.013
		5: 40: 5	78.5	2.19 ± 0.019
		10: 40: 0	119.2	3.33 ± 0.037
Test filtrate III	180.4	1: 40: 9	42.6	1.19 ± 0.035
		5: 40: 5	68.4	1.91 ± 0.018
		10: 40: 0	125.9	3.51 ± 0.024
Test filtrate IV	189.0	1: 40: 9	38.9	1.09 ± 0.012
		5: 40: 5	64.8	1.81 ± 0.047
		10: 40: 0	81.9	2.29 ± 0.053

* TS = test solutions; *i.e.*, the Rhizopus filtrate.

tributing to its production. In each case, however, the yield of *A. niger* was increased with an increase in the amount of the filtrate tested. The above test was repeated twice using the same culture flasks in the same positions. The results were very comparable and showed the same interrelationships as those included in table I. These data suggested that environmental agencies might exert important rôles on the productivity of the stimulant. And since the distribution of the light was not exactly even, this test was repeated in total darkness, in order to investigate this aspect more thoroughly.

II. THE INFLUENCE OF THE WEIGHT OF THE RHIZOPUS MAT ON THE PRODUCTION OF THE GROWTH FACTOR, WHEN CULTURED IN TOTAL DARKNESS

The method of culturing the Rhizopus was the same as that used for test I, save that the culturing took place in total darkness and at 25° ± 1° C. All the filtrates were tested by the standard 25: 5 Aspergillus technique.

These results, like those of test I, fail to show any relationship between the amount of fungal growth and the substance B production. Once again, marked variations occurred between the stimulated powers of several Rhizopus cultures. These variations were repeatable in the same order and degree by using the same flasks in the same positions. Test II tends to eliminate light as the causal agent in the environment. These variations may be caused by a balance between the productions of growth promoting

TABLE II

Rhizopus suinus: THE RELATION OF THE DRY WEIGHT OF MYCELIUM, FOR CULTURES GROWN IN TOTAL DARKNESS, TO THE PRODUCTION OF SUBSTANCE B (5 REPLICAS)

CULTURE	RHIZOPUS: DRY WEIGHT OF MYCELIUM	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE
	mg.	*mg.*	
Control	.....	105.3	1.00
TS–1	49.7	170.0	1.62 ± 0.014
TS–2	53.1	135.1	1.29 ± 0.009
TS–3	63.7	177.4	1.70 ± 0.004
TS–4	49.3	185.2	1.76 ± 0.013

and growth inhibiting factors. The succeeding tests investigate the first hypothesis, and arguments in support of the second hypothesis are presented throughout this investigation and the one to follow (which includes physico-chemical properties of the active factors in the filtrate). The non-correlation noted in the above two tests (I and II) is in accord with the production of substance A by the same species of fungus (BONNER, **1**). These results suggested the advisability of separating substances A and B prior to testing.

III. THE EFFECT OF VARIOUS CONCENTRATIONS OF AN ETHER-TREATED FILTRATE OF RHIZOPUS, ON THE DRY WEIGHT YIELD OF *A. niger*

The Rhizopus was cultured in accordance with the standard method given above. The mat was filtered from the liquid, and the filtrate was layered three times (15 minutes) with equal volumes of peroxide-free ether. Hetero-auxin was demonstrated in the ether-soluble fraction by the accepted pea and oats methods. The aqueous portion, containing ether-insoluble compounds, was tested by the standard *A. niger* method.

TABLE III

DRY WEIGHT YIELDS OF ASPERGILLUS ON VARIOUS CONCENTRATIONS OF ETHER-INSOLUBLE FILTRATES (TS) OF *Rhizopus suinus* (5 REPLICAS)

CULTURE	CONCENTRATIONS TESTED TS: NUTRIENT: H_2O	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE
	ml.	*mg.*	
Control	0: 40: 10	38.0	1.00
TS–1	1: 49: 9	76.8	2.23 ± 0.032
TS–2	2: 40: 8	84.3	2.46 ± 0.068
TS–3	5: 40: 5	115.5	3.36 ± 0.028
TS–4	10: 40: 0	104.3	3.03 ± 0.030

Test III confirms the work of NIELSEN and HARTELIUS (**6**) by which it was demonstrated that the Rhizopus filtrate actually contains two growth

factors which may be separated by their respective solubilities in ether; *viz.*, substance A, or heteroauxin, which is ether soluble; and the ether-insoluble substance B which augments the production of dry matter by *A. niger*.

It is obvious that 5 ml. of the ether-insoluble fraction of the filtrate, in 50 ml. of the test nutrient, yields the maximal results. With the use of untreated filtrate (*cf.* table I), however, much greater concentrations are required before the maximal yield is reached. Since a greater variation in the concentration remains more nearly parallel with the subsequent yield with the use of the untreated filtrate, as compared to the ether-insoluble fraction, the former was used throughout the following tests. Subsequent tests, to be reported later, indicate that the stimulant might be a labile complex and not one definite compound. Therefore, until the definite physico-chemical properties of the so-called substance B are known, the untreated filtrate is used in the tests, since any pre-treatment might produce an indeterminable artifact.

IV. The influence of the liquid area exposed per unit volume on the production of substance B by *R. suinus*

The Rhizopus was cultured in various sized containers so that the area exposed, for 50 ml. of nutrient, varied from 16 sq. cm. to over ten times that area. The inoculum was in proportion to that of the standard method (*i.e.*, $\frac{1}{5}$ the customary amount), and all other conditions were held as heretofore. The various filtrates were tested by the 25:5 Aspergillus method.

TABLE IV

Influence of area exposed per unit volume on the production of substance B (7 replicas)

Culture expressed as area exposed	Rhizopus: dry weight of mycelium	Aspergillus: average dry weight of mycelium	F ± SE
sq. cm.	*mg.*	*mg.*	
Control	.....	247.5	1.00
162.0	133.1	317.1	1.29 ± 0.056
136.9	97.9	270.2	1.10 ± 0.017
99.0	68.5	260.4	1.06 ± 0.016
83.8	42.5	298.9	1.22 ± 0.029
49.5	80.4	351.1	1.43 ± 0.019
31.3	37.0	320.1	1.30 ± 0.017
24.3	33.7	352.5	1.43 ± 0.022
16.0	39.4	290.4	1.17 ± 0.018

The data in table IV fail to reveal any relationship between the areas exposed per unit volume and the effectiveness of the filtrates. These results have two possible interpretations. First, the relative size of the surface area plays no part in the production of substance B. Secondly, the surface area

influences not only the production of substance B, but also of a substance which inhibits the growth of *A. niger*. As a result, the effectiveness of the filtrate would not depend solely on the amount of substance B but rather on the balance of this substance with the inhibitory factors produced in the same culture. The second possibility is the more plausible. It is well known that the presence of inhibitors in substrata on which fungi are cultured is more universal than the occurrence of stimulators. Furthermore, the production of heteroauxin by *R. suinus* parallels the degree to which the cultures are aerated, or to the relative area exposed [BONNER (**1**) and THIMANN (**8**)]. The depression of heteroauxin on the growth of the strain of *A. niger* used was demonstrated by qualitative tests. Heteroauxin was significantly toxic to the strain of *A. niger* employed in concentrations as low as 1 p.p.m., and markedly so at 10 and 100 p.p.m. This is within the range of heteroauxin occurring in *Rhizopus suinus* filtrates. It is, therefore, entirely possible that the surface area did influence the production of substance B, but also affected, in like manner, the production of inhibitors (heteroauxin ?). The untreated filtrates, therefore, failed to show a relationship between the effectiveness and the area exposed, because the stimulating value did not depend on the growth factor alone, but on the balance of this with inhibitory products. Prior to the complete solution of this problem, as well as of other problems relevant to growth factor production, it will be necessary to separate the stimulators from the inhibitors.

V. Effect of white light on the production of substance B by *Rhizopus suinus*

Three Rhizopus cultures were assembled and grown under standard methods, with the following variances. Two flasks were so placed for growth as to be subjected to a bottom white light with an energy value of 570 to 580 ergs/cm.^{2}/sec. The third flask was cultured under similar conditions except that it was kept in total darkness. The respective filtrates were tested by the standard 25 : 5 Aspergillus technique.

TABLE V

EFFECT OF LIGHT AND DARKNESS ON SUBSTANCE B PRODUCTION (3 REPLICAS)

CULTURE	RHIZOPUS: DRY WEIGHT OF MYCELIUM	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE
	mg.	*mg.*	
Control	.....	270.3	1.00
Darkness	99.0	292.4	1.08 ± 0.007
Light 1	32.6	285.0	1.05 ± 0.011
Light 2	96.6	270.3	1.00 ± 0.009

The results in table V indicate little or no effect of bottom light on the production of this growth factor. It is to be noted that one of the cultures exposed to the light produced a filtrate which was ineffective, and that the other produced a filtrate which was less effective than that normally experienced. A comparison of the Rhizopus dry weight yields and the respective values of their filtrates emphasizes once again the lack of correlation between actual growth and the production of the growth factor.

VI. Effect of monochromatic irradiations on substance B production

When employing a light from a top source it is necessary to prevent a focusing effect from the container and any shading from the cotton plugs. This was accomplished by using 12-oz., flat-sided, reagent bottles, which were placed on the broad side under the light whose effect was to be tested. The controls were arranged in a similar position, but placed in total darkness. Because of the size of the containers it was necessary to use 50 ml., rather than 250 ml., of the culture nutrient. All filtrates were tested by the 25 : 5 technique.

The three monochromatic lights were arranged and standardized by Dr. William Gray, to whom sincere thanks are extended. The ultimate light source was a mercury lamp and the monochromatic lights were obtained by combinations of four liquid dye solutions, each 1 cm. in depth. Those used were:

Green ..... Main line at 5640 Å. Intensity 224 to 232 ergs/cm.^{2}/sec. Filter layers: 0.22 per cent. potassium dichromate; 6 per cent. $CuSO_4$; and two of neodymium-NH_4NO_2.

Blue ..... Main line at 4360 Å. Intensity 288 ergs/cm.^{2}/sec. Filter layers: 2 per cent. quinine-HCl; 6 per cent. $CuSO_4$; 0.003 per cent. acid-rhodamine; and ammoniacal-$CuSO_4$.

Yellow ..... Main line at 5780 Å. Intensity 392 ergs/cm.^{2}/sec. Filter layers: 6 per cent. $CuSO_4$; 2 per cent. quinine-HCl; 0.02 per cent. tartrazine + 0.02 per cent. erythrosine; and distilled H_2O.

The effects of the monochromatic lights on the production of substance B are more marked than the effect of the entire visible range. This difference, perhaps, is the result of the direction of the incident light; the white light striking the under side of the mat, and the others the top surface. A more plausible explanation lies in the possibility of opposite effects of the various light ranges tending to balance each other in the complete visible range. This is indicated by the data of table VI. The yellow light greatly reduced the effectiveness of the Rhizopus filtrate as compared with the control cultures in the dark, while the green and the blue lights had the opposite effect.

TABLE VI

EFFECT OF MONOCHROMATIC BLUE, GREEN, AND YELLOW LIGHTS ON THE PRODUCTION OF SUBSTANCE B BY *R. suinus* (7 REPLICAS FOR YELLOW, AND BLUE, 3 REPLICAS FOR GREEN)

CULTURE	RHIZOPUS: DRY WEIGHT OF MYCELIUM	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE
	mg.	*mg.*	
Control 1	.....	228.3	1.00
YELLOW light			
Test 1	15.8	411.1	1.78 ± 0.026
Test 2	22.2	379.0	1.65 ± 0.023
Dark 1	55.2	582.7	2.55 ± 0.108
Dark 2	45.3	487.2	2.14 ± 0.023
BLUE light			
Test 1	31.0	491.4	2.16 ± 0.019
Test 2	26.0	493.7	2.18 ± 0.025
Dark 1	43.4	461.9	2.03 ± 0.028
Dark 2	40.5	442.0	1.94 ± 0.101
Control 2	.....	270.3	1.00
GREEN light			
Test 1	49.1	335.4	1.24 ± 0.021
Test 2	42.0	324.4	1.20 ± 0.011
Dark 1	97.9	299.5	1.11 ± 0.013
Dark 2	80.3	293.1	1.08 ± 0.007

Whether the lights exerted their influences directly on the filtrate or indirectly on the mycelium is not known. All cultures grown in total darkness produced mycelia completely submerged. Those grown in the presence of the lights, however, did develop floating mats with typical aerial hyphae. There was neither sporulation nor pigmentation when subjected to either yellow or blue light. When subjected to green light the sporulation was comparable to that of the white light tests and the pigmentation was but slightly less intense. In these tests, however, there was no direct relationship between the type of growth and the growth factor productivity.

VII. INFLUENCE OF THE SIZE OF THE RHIZOPUS INOCULUM ON THE ULTIMATE PRODUCTION OF SUBSTANCE B

Although the size of the Rhizopus inoculum was varied in neither preceding nor subsequent tests, and therefore played no part in the variations recorded there, it seemed advisable to ascertain whether it influenced the substance B productivity of the cultures. Standard Rhizopus cultures were used except that the size of the inoculum was varied from one-eighth to twice the amount employed in the standard technique.

The data of table VII show that a doubling of the size of the Rhizopus inoculum does result in a significant increase in the stimulative power of the ultimate filtrate. Since the amount of the physiological solution used was

TABLE VII

EFFECT OF THE SIZE OF THE INOCULUM ON THE SUBSTANCE B PRODUCTION BY *Rhizopus suinus* (7 REPLICAS)

CULTURE EXPRESSED AS SIZE OF THE INOCULUM	RHIZOPUS: DRY WEIGHT OF MYCELIUM	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE
	mg.	*mg.*	
Control	.....	356.5	1.00
2 normal	47.0	525.7	1.48 ± 0.013
Normal	54.7	462.1	1.30 ± 0.007
½ normal	48.6	448.2	1.26 ± 0.007
¼ normal	60.0	425.1	1.19 ± 0.007
⅛ normal	51.3	408.1	1.15 ± 0.007

the same for all tests, the inorganic ions thus introduced were not responsible for the results obtained. There are two possibilities of carry-over effects; that in the liquid, and that in the spores themselves. That the former of those two is most probably not the causal agent is indicated indirectly by the fact that increases of Rhizopus filtrates, many fold greater, exert lesser degrees of acceleration. The possibility of a carry-over effect in the spores was not tested. The most acceptable possibility, however, is an indirect one through the effect of the size of the inoculum on the subsequent Rhizopus growth. The greater the concentration of the inoculum, the greater the rate of the initial growth, the earlier the sporulation, and the earlier the initiation of the subsequent autolysis. If substance B production is correlated with the rapidity of growth, with spore production, or with autolysis, this latter hypothesis would tend to explain the results of table VII above. Throughout this series of investigations it was observed that the stimulative power of the filtrate was always greatest in those cultures in which the Rhizopus had formed spores previous to the termination of the run. And, at present, it is tentatively assumed that the last explanation is the most plausible for the interpretation of the results obtained.

VIII. THE RELATIVE PRODUCTIVITY OF SUBSTANCE B, BY *Rhizopus suinus*, AT DIFFERENT TEMPERATURES

Seven standard cultures of Rhizopus were grown at various temperatures. They were placed in available incubators, baths, and rooms whose temperatures fluctuated less than 1° C. The temperatures used were: 11°, 18°, 25°, 31°, 35°, and 41° C. All other conditions were held as nearly equal as possible. The relative humidities and the intensities of the diffused lights, however, varied slightly. The test at 11° C. failed to grow and consequently was not tested. The filtrates of the remaining six cultures were tested by the standard 25:5 Aspergillus technique.

TABLE VIII

EFFECT OF TEMPERATURE ON THE SUBSTANCE B PRODUCTIVITY OF *Rhizopus suinus* (3 REPLICAS)

CULTURE EXPRESSED AS TEMPERATURE	RHIZOPUS: DRY WEIGHT OF MYCELIUM	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE
	mg.	*mg.*	
Control	.....	270.3	1.00
18° C.	88.9	279.5	1.04 ± 0.032
25° C.	109.2	283.7	1.05 ± 0.007
28° C.	200.0	300.5	1.11 ± 0.031
31° C.	771.3	422.3	1.56 ± 0.007
35° C.	128.2	300.0	1.11 ± 0.017
41° C.	43.2	230.4	0.85 ± 0.007

It is obvious that the production of substance B is affected markedly by the incident temperature. Whether this effect is direct or indirect has not been ascertained. Up to and including the optimal temperature of 31° C. there is a relationship between the weight of the Rhizopus mat and the effectiveness of the filtrate. The relationship between the productivity and the abundance of aerial hyphae and sporulation is even more marked. These in outline form are:

18° C.—floating mat; no aerial hyphae; no spores.
25° C.—floating mat; aerial hyphae; few spores.
28° C.—floating mat; aerial hyphae; good spore production.
31° C.—floating mat; aerial hyphae reaching 1 inch in height; very intense sporulation, evident as early as 3½ days.
35° C.—floating mat; aerial hyphae; good spore production.
41° C.—submerged gelatinous mass.

The rapidity with which the mycelia developed was correlated with the degree of sporulation. This, once again, indicates the possibility of a relationship of the production of this growth factor either with the rate of growth or with the process of sporulation. The results obtained at 41° C., however, would require a separate explanation.

NIELSEN (**5**) reported that the maximal yield of substance A (heteroauxin), by the same species, occurred at 35° C. And, BOYSEN JENSEN, investigating the production of heteroauxin by *Aspergillus niger* (**2**), recorded that the greatest accumulation occurred at 36 to 37° C. Their results suggest that the sudden drop in effectiveness noted for the filtrates from cultures grown at temperatures above 31° C. might possibly be due to a marked increase in the production of substance A. The effectiveness tested, therefore, could be the result of the balance of substances A and B. The inhibiting effect of the filtrate of the 41° C. culture is more probably caused by some metabolic product other than heteroauxin, since the production of this

substance is known to be very low at the higher temperatures. Although the results obtained are not necessarily indicative of the absolute productivity of substance B, they do show that the stimulative value of the Rhizopus filtrate, as tested by *Aspergillus niger*, is influenced markedly by the temperature. The cardinal temperatures, in relation to the effectiveness of the filtrate, are: minimal, near 18° C.; optimal, 31° C.; maximal, slightly above 35° C. It is assumed, however, that the production of substance B parallels this effectiveness relatively closely.

IX. Aeration of the Rhizopus culture and its effect on the substance B production

Six standard Rhizopus cultures were used for these tests. One was plugged with cotton wadding as used throughout all other tests (solution K). The second flask was closed with a rubber stopper and sealed with paraffin (solution Seal). The third culture was aerated with sterilized air bubbling through the solution at the rate of six bubbles per minute (solution Air). The fourth was the same as the third, save nitrogen was substituted for the air (solution N). In the fifth, pure oxygen replaced the air of the third (solution O–1). And in the sixth, the space above the solution was aerated with pure oxygen at the same rate (solution O–2). Other cultural procedures were as of the standard technique, and the untreated filtrates were tested by the 25 : 5 method.

TABLE IX

Effect of aeration on substance B production (3 replicas)

Culture	Rhizopus: dry weight of mycelium	Aspergillus: average dry weight of mycelium	F ± SE
	mg.	*mg.*	
Control*	.....	100.8	1.00
Seal†	144.0	145.5	1.45 ± 0.028
K*	149.1	160.2	1.59 ± 0.015
O–2	300.2	170.2	1.65 ± 0.043
Air	173.7	174.1	1.73 ± 0.056
O–1	174.5	206.5	2.06 ± 0.045
N	No growth	.....	....

* Four replicas.
† Submerged mat.

The production of substance B is directly related to the degree of aeration, as is illustrated by the results shown. This increase in substance B accumulation, resulting from increased aeration, is undoubtedly more marked than the above data indicate, since Bonner (1) showed that heteroauxin production, by the same species, is greatly increased by an increase in aerobic metabolism, and since preliminary tests demonstrated the toxicity of this substance to the growth of *Aspergillus niger*.

The results obtained for the sealed culture show that the flask contained ample air, not only for growth but for a significant production of substance B. Although, in most cases, the production of this growth factor appeared to possess a relationship with rapid initial growth and with spore formation, these same results demonstrate that the process of asexual fruiting is not absolutely essential. A continuous exchange of the air above the liquid (O–2) resulted in a greater effectiveness of the filtrate, while the stimulative value of the filtrate of the flask stoppered with cotton wadding was intermediate to these last two. Herein lies a partial explanation of the variations in effectiveness observed for various cultures grown under strictly standard methods. The tighter the wadding the less the degree of gaseous exchange, and therefore a decrease in the production of the growth factor. Complete aeration of the liquid and of the gas above it (O–1) resulted in a 50 per cent. increase in production over that obtained for the sealed culture. Since the Rhizopus failed to grow under anaerobic conditions, and since growth is a prerequisite to substance B production it was impossible to ascertain whether oxygen is essential for the formation of this growth factor.

The data above, once again, demonstrate the lack of a relationship between the mycelial production of Rhizopus and the amount of the substance B produced.

X. Effect of filter paper on the production of substance B by *Rhizopus suinus*

It has been reported that filter paper not only influences the accumulation of the heteroauxin formed, but also that it stimulates the growth of this fungus (Worley, **10**). It seemed advisable, therefore, to test whether filter paper would play a similar rôle in substance B production. Two flasks of the fungus were cultured; the one was run under standard culture methods, while the other contained one shredded 120-mm. filter paper. The filtrates were concentrated to one fifth their original volumes, by partial evaporation, and then diluted to the concentrations desired. The following concentrations were prepared and tested.

F–5.0 = 1 ml. of concentrate equivalent to 5.0 ml. of filter-paper filtrate.
F–2.5 = 1 ml. of concentrate equivalent to 2.5 ml. of filter-paper filtrate.
etc.
W–5.0 = 1 ml. of concentrate equivalent to 5.0 ml. of regular filtrate.
W–2.5 = 1 ml. of concentrate equivalent to 2.5 ml. of regular filtrate.
etc.

The filtrates were tested not only by the *Aspergillus niger* method (table XI), but also by the *Colletotrichum circinans* method reported by Worley and Duggar (**11**). In lieu of the diameter measurements, as given in their paper, the comparisons were made on total areas (table X). This change

permits a comparison with dry weight measurements of fungi grown on liquid cultures. The results are also given in a form to show the relative effects of the test solutions at different time periods. In addition to the two accepted methods of interpretation, *viz.*, radial, and ring-area, the sector-area method was employed. The advantages of this latter method have been given by WORLEY (9).

TABLE X

EFFECT OF FILTER PAPER ON SUBSTANCE B PRODUCTION, AS TESTED BY THE *Colletotrichum circinans* METHOD (5 REPLICAS)

CULTURE	TOTAL AREAS (SQ. CM.) OF *Colletotrichum circinans* AT VARIOUS TIME INTERVALS							
	0	3 DAYS	6 DAYS	8 DAYS	11 DAYS	14 DAYS	17 DAYS	20 DAYS
Control	0.008	0.33	2.75	5.30	10.18	15.55	22.90	31.47
F–5.0	0.008	0.61	3.73	7.65	13.85	22.06	32.17	42.78
F–2.5	0.008	0.55	3.63	6.33	13.20	21.24	30.58	41.06
F–1.5	0.008	0.52	3.56	6.20	12.32	19.24	27.81	37.18
F–1.0	0.008	0.57	3.63	6.29	12.76	19.87	28.27	38.26
F–0.5	0.008	0.65	3.70	6.68	12.88	19.01	27.34	35.26
W–5.0	0.008	0.54	3.56	6.25	12.25	19.48	28.56	38.48
W–2.5	0.008	0.40	2.52	4.83	10.24	17.20	26.15	35.78
W–1.5	0.008	0.31	2.81	5.68	11.70	18.70	28.37	37.50
W–1.0	0.008	0.54	3.37	5.94	12.38	19.32	27.25	37.39
W–0.5	0.008	0.54	3.43	6.16	12.44	19.32	25.97	33.29

The same test solutions were diluted to one part in five and then tested by the 25 : 5 *Aspergillus niger* method. The results are given in table XI.

TABLE XI

EFFECT OF FILTER PAPER ON SUBSTANCE B PRODUCTION, AS TESTED BY *Aspergillus niger* (3 REPLICAS)

CULTURE	ASPERGILLUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE
	mg.	
Control	52.3	1.00
F–2.5	217.0	4.16 ± 0.062
F–1.5	207.9	3.98 ± 0.068
F–1.0	184.2	3.53 ± 0.055
F–0.5	138.4	2.65 ± 0.149
W–2.5	362.3	6.94 ± 0.219
W–1.5	231.8	4.44 ± 0.086
W–1.0	192.9	3.69 ± 0.061
W–0.5	173.8	3.31 ± 0.044

The interpretation of the effect of filter paper, in the nutrient solution, on the production of substance B is dependent upon the test organism used. The above data show a significantly higher augmentation of the growth of *Colletotrichum circinans* on the addition of a filter-paper filtrate than on

the addition of a regular filtrate. This increase in acceleration, arising from the presence of the filter paper, is significantly greater regardless of the concentration of the filtrate used. It is of interest to note that the lower concentrations of the test filtrate frequently show a greater augmentation during the earlier stages of growth, while increasing amounts give parallel increases of growth during the later stages. Throughout these tests, however, the regular filtrate (without filter paper) tended to show a lesser stimulation than the other at the same concentration. That a tenfold concentration did not show an increase in growth ten times as great, and the fact that the less concentrated filtrates show a greater acceleration during the earlier stages are indicative of other factors being limiting in effect on the utilization of superoptimal amounts of substance B by *Colletotrichum circinans*. The results of the higher concentrations are given in graphic form (fig. 1) to

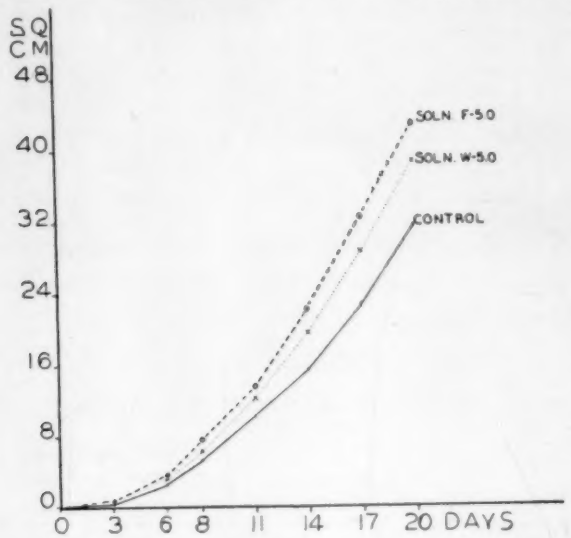

FIG. 1. The effect of filter paper on the production of substance B, as tested by *Colletotrichum circinans* (discussion in text).

show more clearly the difference of effects between the regular and the filter-paper filtrates upon this organism. Both the data of table X and of figure 1 indicate a steady acceleration of the tests over the control throughout the entire duration of the runs. That the above is not the case is shown by the calculations of ratios, one set of which is given in graphic form as figure 2. It becomes obvious that the growths of the test cultures, as compared with the control culture, are very erratic, and that the more stimulative the filtrate the greater is its variability. Most of the tests show a marked stimulating effect during the first three days, an inhibiting one during the next several,

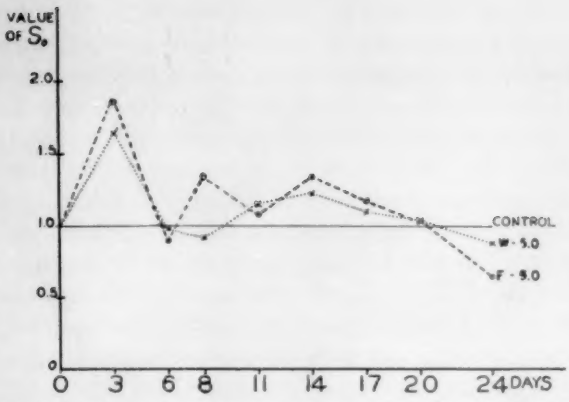

FIG. 2. The irregularity of stimulation at various time intervals (discussion in text).

a second and lesser augmentation up to approximately the seventeenth day, no effect near the twentieth day, and a slight inhibition thereafter. No explanation is offered for these marked variations in the degrees of acceleration during the various periods of growth.

These same factors were tested by a second organism, *viz.*, *Aspergillus niger*. The data of table XII reveal that the effectiveness of the test filtrates upon this fungus was the reverse of that shown by *Colletotrichum circinans*. In this case, the regular filtrates showed a marked and consistently higher stimulating effect than those of the filter paper.

This difference in effectiveness, as shown by the two test organisms, is further proof of the presence of both inhibiting and stimulating factors. It is also possible that the two test organisms are stimulated by two different metabolic products. Such an explanation, however, is not plausible. This is also shown by recent work which indicates a greater and greater similarity of growth factors for the various microorganisms. It is tentatively assumed that the effectiveness is dependent upon the balance of stimulators and inhibitors, and that a given balance may affect two test organisms in different manners. If it is assumed that the inhibitor and the stimulator mutually influence *Aspergillus niger* when present in the same concentrations, then the inhibitor (heteroauxin ?) must accumulate in greater concentrations in the presence of filter paper than does the growth factor. If this assumption is true, the effect of the factor on *Colletotrichum circinans* must be more potent than the inhibitor. The results with *Aspergillus niger* alone could be interpreted as showing that filter paper augments the accumulation of an inhibitor and plays a lesser or no part in the accumulation of the factor. The results with *Colletotrichum circinans* are then indicative of different growth regulators or are proof that the above mentioned condition does not exist. On the other hand, if there are but two substances, and if the effec-

tiveness of these two contrasting factors are different reciprocally, their respective accumulations cannot be hypothesized from the data at hand. In any case, however, a given balance of the two contrasting factors must affect the two organisms in different manners. A fourth explanation is possible. The filter paper itself may contain a soluble substance stimulative to *Colletotrichum circinans* and another inhibitive to *Aspergillus niger*. These substances may have been carried over in the filtrates and thus exert an effect on the organisms. The concentration of any soluble substance of the filter paper in the ultimate test solution, however, would be so small that this possibility is of little value. Furthermore, all solutions were filtered with the same type of filter paper, thereby permitting the accumulations of any such hypothetical substances in both types of test filtrates. Regardless of the explanation accepted, the first being the more plausible, the addition of filter paper results in an increased acceleration for *Colletotrichum circinans* and a decreased augmentation for *Aspergillus niger*.

General discussion and summary

Few studies in the metabolism of microorganisms have dealt with the quantitative production of the so-called growth factors. Such investigations have been limited by the lack of sufficiently specific tests. Until these factors have been isolated or at least freed from concurrently occurring inhibitors and other stimulators, and until either biological or chemical tests have been developed to measure a specific compound microquantitatively, it will be impossible to ascertain the effect of the environment or the composition of the substratum on the quantitative production of growth factors by these lower organisms. Therefore, present studies are *nothing more than indications in induced alterations in the effectiveness* of a solution or tissue. They result in but a poor insight into the changes in effectiveness and fail to prove definitely whether the alteration is due to a change in the absolute amount of the growth factor or to a change in the balance between this factor (and other stimulators) and the inhibitors. Throughout this discussion it is to be remembered that the effects stated are those on effectiveness rather than on substance B itself.

A survey of all tests run demonstrates the lack of a correlation between the amount of the growth factor produced (*i.e.*, total effectiveness) and the amount of *Rhizopus suinus* mycelia contributing to its production. Certain tests, however, do indicate a relationship of substance B production with the type of growth. Either a rapid initial growth or an intense spore production by the Rhizopus results in a greater accumulation of the factor in its filtrate. Although most tests show a relationship between sporulation and substance B production, other results show that the fructifications are not a prerequisite for the formation of the factor. It is also possible that these

two types of growth are indicative of an early autolysis, and that it is this latter process which liberates the active principle into the liquid substratum.

No relationship exists between the areas exposed per unit volume and the effectiveness of the respective filtrates, even though the surface areas vary over a tenfold range. Since the work of others shows that the production of heteroauxin by *Rhizopus suinus* is dependent upon the surface area, and since this substance acts as an inhibitor to the test organism, it is plausible that the production of substance B is affected in like manner and that the constant effectiveness of the filtrates indicate rather the lack of change in the balance between stimulators and inhibitors.

White light exerts little or no effect on substance B formation. The effects of monochromatic lights, on the other hand, were more marked. Yellow light reduces greatly the effectiveness of the Rhizopus filtrate, while green and blue lights have the opposite effect. No tests were conducted to determine whether these effects were direct or indirect through the type of growth induced.

A doubling of the size of the Rhizopus inoculum resulted in a significant increase in the effectiveness of the ultimate filtrate. This effect is most probably due to a more rapid initial growth followed by early sporulation and subsequent autolysis.

Changes in temperature have the most marked effect of any agent tried. The cardinal temperatures for the production of this factor are: minimal, near 18° C.; optimal, 31° C.; maximal, 35+° C. These effects are probably indirect through the type of growth or are the result of the effective balance of substance B and heteroauxin productions.

One set of tests demonstrated that the production of substance B is directly related to the degree of aeration.

One run tested the effect of filter paper on the growth factor accumulation. If tested by *Colletotrichum circinans* there was an acceleration in the production, while if tested by *Aspergillus niger* there was a slight decrease. Four possible explanations were given.

The variations noted for replicas of Rhizopus control cultures are possibly due to additive effects of very slight variations in size of the inoculum, degree of aeration (*i.e.*, tightness of wadding), the incident light, and the temperature.

Sincere thanks and appreciation are extended to Dr. B. M. Duggar, of the University of Wisconsin, under whom this work was conducted and the protocol of this paper prepared.

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LITERATURE CITED

1. BONNER, J. The production of growth substance by *Rhizopus suinus*. Biol. Zentralbl. **52**: 565–582. 1932.
2. BOYSEN JENSEN, P. Über Bildung eines Wachstumsregulators durch *Aspergillus niger*. Biochem. Zeitschr. **239**: 243–249. 1931.
3. ————————. Über die Bildung und biologische Bedeutung des Wachstumsregulators bei *Aspergillus niger*. Biochem. Zeitschr. **250**: 270–280. 1932.
4. KOSER, S. A., and SAUNDERS, F. Accessory growth factors for bacteria and related microorganisms. Bact. Rev. **2**: 99–160. 1938.
5. NIELSEN, N. Untersuchungen über einen neuen wachstumsregulierenden Stoff: Rhizopin. Jahrb. wiss. Bot. **73**: 125–191. 1930.
6. ————————, and HARTELIUS, V. The separation of growth promoting substances. Compt. Rend. Lab. Carlsberg **19**(8): 1–17. 1932.
7. PESKETT, G. L. Growth factors of lower organisms. Biol. Rev. **8**: 1–45. 1933.
8. THIMANN, K. V. On the plant growth hormone produced by *Rhizopus suinus*. Jour. Biol. Chem. **109**: 279–291. 1935.
9. WORLEY, C. L. Interpretation of comparative growths of fungal colonies growing on different solid substrata. Plant Physiol. **14**: 589–593. 1939.
10. ————————. *Rhizopus suinus* Niel. and growth factors. Thesis, Univ. of Wisconsin. 1939.
11. ————————, and DUGGAR, B. M. *Colletotrichum circinans* as a semi-quantitative test unit for the growth substance produced by *Rhizopus suinus*. Science n.s. **88**: 132. 1938.

EFFECTS OF pH AND THE COMPONENTS OF BICARBONATE AND PHOSPHATE BUFFERED SOLUTIONS ON THE METABOLISM OF POTATO DISCS AND THEIR ABILITY TO ABSORB IONS[1]

F. C. Steward and C. Preston

(WITH NINE FIGURES)

Introduction

This paper consists of two parts. The first concerns the effect of certain variables on the absorption of bromide by potato discs and experiments which were made by the senior author in the Division of Plant Nutrition, University of California, in 1933–1934. The second part describes the effect of similar treatments on the metabolism of potato discs and experiments carried out with the assistance of the junior author at Birkbeck College, London, in 1937–1938. Since the results of the later work are essential for a full interpretation of that which preceded it, the two investigations are described together.

Investigation of the effects of external pH presents an obvious approach to the problem of salt accumulation. It is, therefore, an essential part of any survey of the variables which affect this important process (**18, 19, 20**). Despite the great emphasis which others have placed upon the rôle of pH in salt absorption, detailed discussion of the effect of this variable on bromide absorption by potato discs has been long postponed. For some preliminary observations see (**18**). This delay was due to the knowledge that until the effects of a wide range of variables—including the nature and concentration of neutral salts—upon the behavior of cells had been investigated, phenomena due to hydrogen ion *per se* could not be segregated from those due to other variable components of the system used to control pH. Knowledge of the metabolic processes of potato tissue and the way these are affected by neutral salts (**21, 22, 23**) is now adequate to permit investigation of the problem.

Of the buffer systems which can be used to regulate the pH of solutions in contact with plant cells, that which depends upon the proportion of bicarbonate to free carbonic acid is of outstanding interest since its components are metabolic products of cells. This paper contains a survey of the effects of pH, carbon dioxide, and potassium carbonate concentration. Though somewhat incomplete, it enables us to visualize the trend of the effect of these variables upon absorption and metabolism of potato discs and prepares

[1] This paper is the fourth of a series of papers on the biochemistry of salt absorption by plants. The writers are indebted to Prof. D. R. Hoagland for proofreading this paper.

the way for the rather formidable task which a still more complete investigation entails. A similar survey made with phosphate—buffered solutions shows the extent to which the effects obtained in bicarbonate solutions are peculiar to a particular buffer system.

Methods

Potato discs were used under the standard conditions which have proved conducive to salt accumulation. Bromide was used as an indicator of anion absorption and was supplied as potassium bromide at the same equivalent concentration throughout a series of experiments. All known variables, other than salt concentration and pH, which affect salt absorption were standardized as follows: temperature 23° C., mean disc thickness, 0.75 mm., number of discs in two liters of solution which was stirred (100 r.p.m.) and aerated (total gas flow 15 liters per hour) to maintain equilibrium with the oxygen tension in the gas used. This facilitated comparisons between a series of experiments which, though not run concurrently, were carried out in rapid succession using tissue from the same uniform stock of tubers. A standard experimental time of 70 or 72 hours was used.

The experiments were of two general kinds: experiments at constant salt (potassium bicarbonate or phosphate) concentration but embracing a range of pH values; and experiments at constant pH in which the total salt (potassium bicarbonate or phosphate) was the variable. At constant potassium bicarbonate concentration, the range of pH values was obtained by changing the partial pressure (composition by volume at constant pressure) of carbon dioxide in the gas stream which flowed through the culture vessels. For the comparable procedure in the phosphate series free acid (H_2SO_4 or H_3PO_4) was added to solutions of potassium phosphate (K_2HPO_4) as required. To reveal the specific effects due to these buffer salts (potassium bicarbonate and phosphate) without confusion with those due to pH, the total salt concentration was varied at *constant pH*. To eliminate confusion between the effects due to the free acid or anion and those merely due to the cation (K), certain experiments were also carried out at constant potassium concentration. In the latter case a low concentration of the experimental salt (potassium bicarbonate or phosphate) was raised to the desired potassium level by the addition of the required amount of a salt (potassium sulphate) in which the anion has been shown (**22**) to be without conspicuous effects on the metabolism of potato discs.

The required partial pressure of carbon dioxide was obtained by combining streams of oxygen and carbon dioxide at known rates of flow. Oxygen was used in order that even the richest mixture (26.6 per cent. CO_2) did not contain an oxygen pressure so low that behavior of the tissue would be limited thereby. Manifolds were connected to a high pressure source of

oxygen which delivered the *total* flow required at a constant rate which was regulated by a reducing valve. A similar device was also used for carbon dioxide. For each gas mixture, the flow of oxygen and carbon dioxide, drawn off at convenient points on the manifolds, was regulated by separate needle valves which were adjusted in accordance with the readings of a calibrated flow meter (**15**) through which the mixture passed. Thorough mixing occurred in the large pressure stabilizers which were described with the original apparatus (**15**). The final adjustment of the needle valves was so made that the gas mixture passing through a wash bottle which contained the required bicarbonate concentration, gave the requisite pH as seen by the indicator which was added for the purpose. The final record of the pH of the salt solutions actually in contact with the tissue was made by a glass electrode, but the indicator in the wash bottle gave warning if the mixing apparatus was not working faithfully. In this way the reaction of solutions was kept within narrow limits throughout the period of experiments. The volume percentage of carbon dioxide in the gas mixture used was recorded by gas analysis in a modified type of HEMPEL's apparatus.

Results

THE EFFECT OF pH, POTASSIUM BICARBONATE, AND DISSOLVED CARBON DIOXIDE ON BROMIDE ABSORPTION

The combined results of three experiments are assembled in table I. The choice of the concurrent treatments was determined partly by convenience in operation and partly by considerations which need not concern us here; it is the impression which the *combined* results convey of the effect of pH, added bicarbonate, and carbon dioxide on the accumulation of bromide which is of interest.

At constant bromide concentration, in the absence of added bicarbonate and in solutions in equilibrium with a gas free of carbon dioxide, the bromide uptake was not significantly affected by the extreme range of total potassium concentration which these experiments incurred. This factor can thus be ignored. It is very clear, however, that the components of the system which did affect bromide absorption profoundly were the concentration of hydrogen ions, bicarbonate, and carbon dioxide. It remains to be seen which of these is the most important.

At any given pH (pH 7.23 and 6.8) the effect of a simultaneous increase of bicarbonate and dissolved carbon dioxide is to greatly reduce the bromide absorbed; so much so, in fact, that by such increases alone the *accumulation* of this ion *was almost entirely suppressed* even though all other variables were favorable. Ignoring for the moment the differences in pH (7.2 and 6.8) at which two different experiments were conducted, it can be shown that over the range of added bicarbonate concentrations (0.0 to 0.020 equiv.

TABLE I

EFFECT OF pH, [$KHCO_3$] AND [CO_2] ON ACCUMULATION OF BROMIDE BY POTATO DISCS FROM 0.001 EQUIVALENTS PER LITER KBr AT 23° C. DURING 70 HOURS

EXPERIMENT*	[$KHCO_3$] EQUIV. PER LITER	PERCENTAGE CO_2†	MEAN pH OF EXTERNAL SOLUTION	[Br] SAP. PER LITER	EXPERIMENT	[$KHCO_3$] PER LITER	PERCENTAGE CO_2†	MEAN pH OF EXTERNAL SOLUTION	[Br] SAP. PER LITER	EXPERIMENT	[$KHCO_3$] PER LITER	PERCENTAGE CO_2†	MEAN pH OF EXTERNAL SOLUTION	[Br] SAP. PER LITER
		%		*mg. equiv.*		*mg. equiv.*	%		*mg. equiv.*		*mg. equiv.*	%		*mg. equiv.*
1	–	26.6	4.75	7.88	1	0.001	26.6	5.3	5.26	1	–	0.0	6.90	17.3
										3	–	0.0	7.70	17.3
2	–	7.8	5.92	14.3	3	0.001	.....	6.8	15.3	2	0.0050	4.2	7.23	11.5
										2	0.0100	7.8	7.23	6.8
2	–	4.2	6.12	16.1	2	0.001	0.8	7.3	15.8	3	0.0025	2.2	6.81	14.1
2	–	0.8	6.58	16.5	3	0.001	.....	7.7	15.9	3	0.0150	12.5	6.76	2.9
1	–	0.0	6.90	17.3	1	0.001	0.0	8.3	16.1	3	0.0200	16.5	6.81	1.7
3	–	0.0	7.70	17.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

* Expt. 1. Total [K] = 0.002 equiv. per liter
Expt. 2. Total [K] = 0.011 " " "
Expt. 3. Total [K] = 0.021 " " "

† Volume percentage CO_2 in gas phase.

per liter) the relationship between bromide absorbed by the discs and either the external potassium bicarbonate concentration (fig. 1) or the volume percentage of carbon dioxide in the gas phase, is smooth. At pH 7.2 and 6.8 the curves are almost coincident and are linear over much of the range of bicarbonate (0.0 to 0.010 equiv. per liter) or carbon dioxide concentrations (0 to 8.0 per cent. by vol.) although they do deviate at higher bicarbonate concentrations from the straight line as they tend to approach somewhat asymptotically the bromide concentration of the external solution. It is, therefore, clear that some component of the system which is increased proportionally to the added bicarbonate or the dissolved carbon dioxide depresses the bromide uptake. To fully map out the depressant effect of bicarbonate and dissolved carbon dioxide at each pH value would be a considerable task. It suffices for present purposes, however, to identify by a straight line the slope of the curve which expresses this relationship and, extrapolating this to zero bromide concentration, slight error will be encountered only at the highest concentrations of bicarbonate.

In the absence of added bicarbonate, increased pressure of carbon dioxide in the gas phase caused lower pH in the solution; when it became more acid than pH 6.9, the bromide absorption was decreased—especially so below a critical value of approximately pH 6.0—when the decrease was rapid (fig. 1). As shown by paired cultures which received the same gas mixture, the

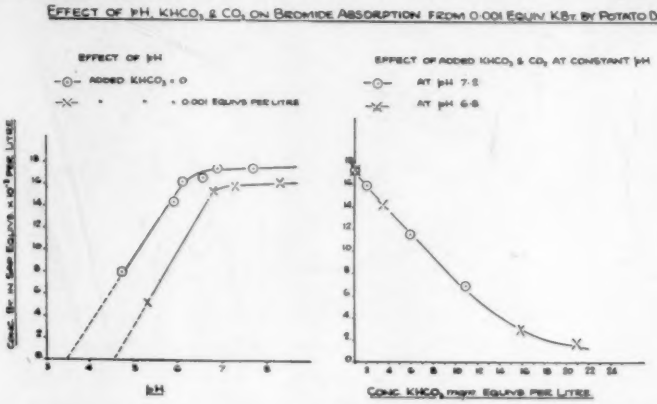
EFFECT OF pH, KHCO₃ & CO₂ ON BROMIDE ABSORPTION FROM 0·001 EQUIV KBr BY POTATO DISCS AT 23°C
EFFECT OF pH
ADDED KHCO₃ = 0
0·001 EQUIVS PER LITRE
pH
EFFECT OF ADDED KHCO₃ & CO₂ AT CONSTANT pH
AT pH 7·2
AT pH 6·8
CONC. KHCO₃ (milli- EQUIVS PER LITRE

FIG. 1.

dissolved carbon dioxide, free to exert its unbuffered effect on pH, depressed bromide absorption much less than did the combined effect of dissolved carbon dioxide and added bicarbonate at more alkaline reactions. To appreciate the full range of effects of bromide absorption (70 hr. at 23° C. from 0.001 equivalents KBr per liter), the effect of pH and, at each pH, of increased bicarbonate and carbon dioxide concentration must be visualized. This can be done if three co-ordinates (bromide concentration in sap after

70 hr., pH of external solution, bicarbonate concentration in the solution or percentage by volume of carbon dioxide in the gas) are plotted simultaneously (fig. 2). This method of presentation will be used throughout, as it permits the effects of several variables to be visualized simultaneously.

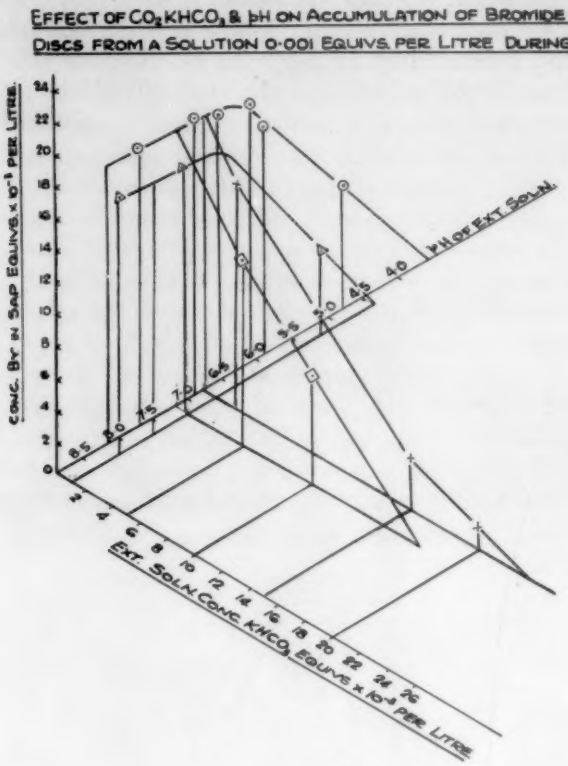
EFFECT OF CO_2 $KHCO_3$ & pH ON ACCUMULATION OF BROMIDE BY POTATO
DISCS FROM A SOLUTION 0·001 EQUIVS. PER LITRE DURING 70 HOURS AT 23°C.
CONC. Br IN SAP EQUIVS. x 10^{-3} PER LITRE.
pH OF EXT. SOLN.
EXT. SOLN. CONC. $KHCO_3$ EQUIVS. x 10^{-4} PER LITRE.

FIG. 2.

To specify completely the relationships of bromide uptake to pH in bicarbonate buffered solutions the surface of a solid model is required. By making the fullest use of the data available, it is possible to visualize the kind of surface which expresses these relationships. The solid model may be constructed in two different ways and each serves a useful purpose.

THE EFFECT OF pH AND ADDED $KHCO_3$ ON BROMIDE UPTAKE.—In the simplest procedure, the two variables plotted are those which were set at arbitrary values in the actual experiments; namely, pH, and the concentration of potassium bicarbonate added to the solution. The third is the concentration of bromide observed in the sap after a 70-hour treatment. One surface of such a model (fig. 3) can be specified at once from the data at zero concentration of added potassium bicarbonate. At each pH, a section

through the model parallel to the bicarbonate concentration axis is bounded by the line, assumed for this purpose straight, which records the rate of *decrease* of bromide uptake with increased bicarbonate and carbon dioxide concentration. A sufficient number of these lines enable the surface of the complete model to be visualized.

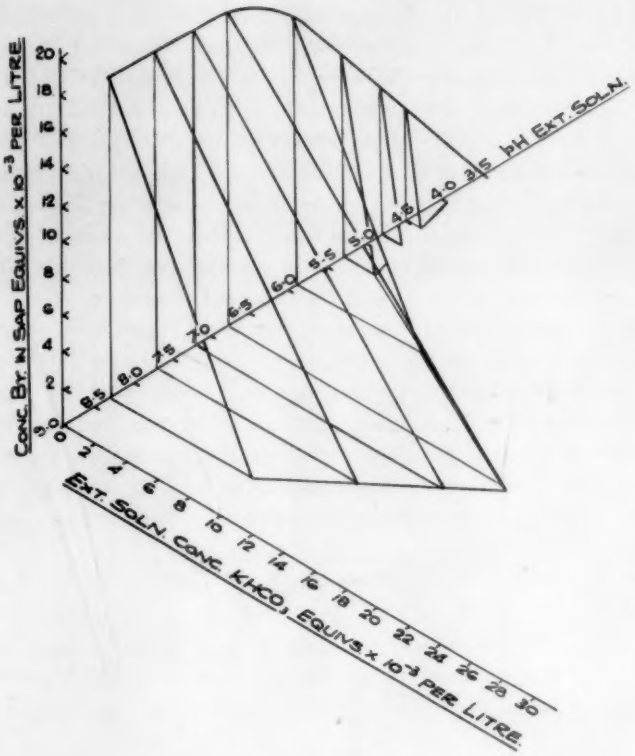
EFFECT OF CO_2 $KHCO_3$ & pH ON ACCUMULATION OF BROMIDE BY POTATO DISCS FROM A SOLUTION 0·001 EQUIVS PER LITRE DURING 70 HOURS AT 23°C.
CONC. BY IN SAP EQUIVS x 10^{-3} PER LITRE
0 2 4 6 8 10 12 14 16 18 20
8·5 8·0 7·5 7·0 6·5 6·0 5·5 5·0 4·5 4·0 3·5 pH EXT. SOLN.
EXT. SOLN. CONC. $KHCO_3$ EQUIVS x 10^{-3} PER LITRE
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

FIG. 3.

Figure 3 shows that, in the absence of added bicarbonate there is a considerable range (pH 8.0 to 6.0) in which pH and dissolved carbon dioxide concentration have but little effect on bromide absorption. That a hundredfold range of hydrogen ion concentration is without conspicuous effect on bromide uptake is in agreement with other results (**6, 18,** p. 1012) which show that salt absorption may be less dependent upon pH than upon other

variables. At reactions more acid than pH 6.0, bromide absorption was reduced and its decline with acidity implies that at *about pH 3.5 bromide absorption should vanish* (fig. 3). At increased bicarbonate concentration (0.001 equiv. per liter) bromide uptake was decreased at all reactions (table I; figs. 2, 3) and from the graph (fig. 2) it is clear that bromide uptake should vanish at a much less acid reaction (pH 4.5) in presence of 0.001 equivalents of bicarbonate.

Having fixed the reactions (pH 3.5 and 4.5) at which bromide uptake vanishes at zero concentration of bicarbonate and at 0.001 equivalents per liter, it can be estimated with sufficient accuracy for the intermediate concentrations. The bromide concentration attained in the sap from solutions at pH 5.3 and two different bicarbonate concentrations can be found, and these values fix the steep slope of the bromide uptake-bicarbonate concentration curve which intercepts the plane of zero bromide uptake (fig. 2) at a bicarbonate concentration of 0.002 equivalents per liter. At pH 6.8 and 7.2 the data available fix the slope of the curve which represents the effect of bicarbonate and carbon dioxide on bromide uptake (fig. 2) with some certainty; and by joining points at which bromide absorption vanishes, the probable boundary of the model is thus ascertained. At each of the two reactions (pH 7.7 and 8.3) data on bromide uptake are available for one bicarbonate concentration only. It can be obtained, however, at the other by reference to the well defined curves of figure 2. Although these two points represent the minimum to establish the slope of the line passing through them, these lines have been prolonged in figure 3 to the plane of zero bromide uptake. It will be shown later that the resultant figure and the curve in which it cuts the plane at zero uptake—a curve which specifies the conditions of pH and bicarbonate concentration which suppress bromide absorption—can be correlated with the metabolism of potato discs under a similar set of conditions.

The characteristics of this model which are of interest are as follows: At constant pH the depression in the bromide absorption which is due to an increase in potassium bicarbonate concentration is a minimum at a pH of 6.8; *i.e.*, uptake is at a maximum. This reaction is near to strict neutrality and also to the pK value of carbonic acid as a monobasic acid. At pH = pK, (pH = 6.4) the ratio $\frac{\text{salt}}{\text{free acid}} = 1$. At reactions more acid than this the amount of free acid becomes rapidly much greater than the salt added; at reactions more alkaline than pH = 6.4 the free acid decreases until at pH 8.3 it is virtually zero. Since there is a greater effect of added bicarbonate at constant pH at *both* more acid and more alkaline reactions than pH = 6.4, the effective component of the system cannot be immediately selected from all those which are affected by increased bicarbonate concentration. It is

suggestive that at acid reactions where the unneutralized carbonic acid exceeds the salt present, the effect of increased bicarbonate concentration at constant pH is particularly great.

THE EFFECT OF pH AND TOTAL CARBONIC ACID ON BROMIDE ABSORPTION.—In figure 4, the variables plotted are bromide concentration in the sap (mg. equiv. per liter), pH of the external solution, and total carbonic acid in the solution; *i.e.*, that which is present as salt ($KHCO_3$) *plus* the uncombined acid. For the bicarbonate solutions the free acid can be calculated from the relation $pH = pK_1 + \log \frac{[salt]}{[free\ acid]}$ where $pK_1 = 6.4$ and in the absence of potassium bicarbonate the total dissolved carbonic acid is given by the relationship: mols H_2CO_3 per liter $= 3.645 \times 10^{-2} \times P$, where P = partial pressure in atmospheres (volume percentage of $CO_2 \times$ total barometric pressure in atmospheres) of carbon dioxide in the gas phase. The data are given in table II.

In figure 4, data from experiments at the same concentration of *added*

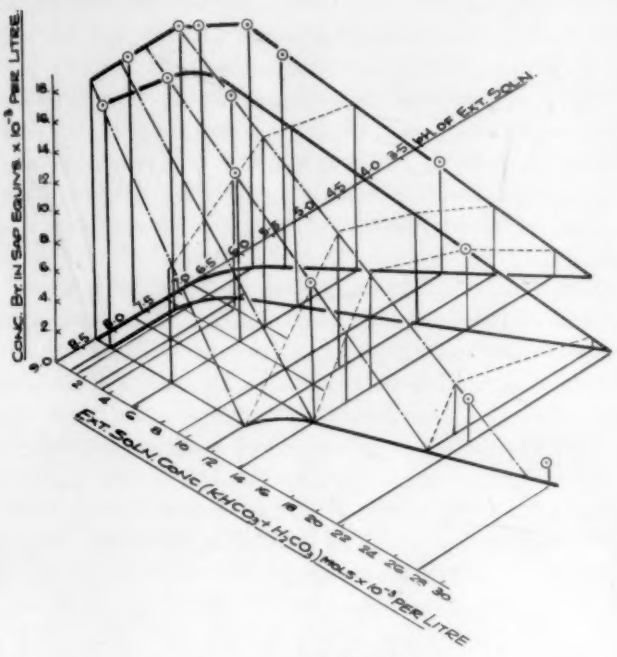
EFFECT OF CO_2 $KHCO_3$ & pH ON ACCUMULATION OF BROMIDE BY POTATO DISCS FROM A SOLUTION 0·001 EQUIVS. PER LITRE DURING 70 HOURS AT 23°C.
CONC. BR. IN SAP EQUIVS. × 10^{-3} PER LITRE.
pH OF EXT. SOLN.
EXT. SOLN. CONC. ($KHCO_3$ + H_2CO_3) MOLS. × 10^{-3} PER LITRE

FIG. 4.

potassium bicarbonate all lie in a curved surface which cuts the basal plane of the model in a curve. This curve which traces out the increase in the total carbonic acid as a given concentration of bicarbonate (initially at pH 8.3), is brought to more acid reactions by dissolved carbon dioxide. Such a curve, at zero added potassium bicarbonate, defines one boundary of the model; another, at 0.001 equivalents of added $KHCO_3$ per liter, has been drawn. At points on these curves, indicated by the thin construction lines, ordinates have been erected which correspond with the experimentally observed bromide concentration in the sap. The several data at pH 7.2 and 6.8, respectively, define two vertical sections cut through the model at these pH values[2] and the edge of the section thus exposed (which can be treated as linear) represents the *depressing effect at constant pH of total dissolved carbonic acid on bromide uptake*. In this way two curves and two straight lines, which lie in the surface of the model, can be defined. At any given *total* carbonic acid concentration vertical sections[3] can be cut through the model (at right angles to the sections at constant pH) and ordinates erected to intercept the curves which lie in the surface and have been defined above. Observed data at pH 7.5 and 8.3 were insufficient to establish the section along these planes of constant pH value but, by prolonging to pH 7.5 and 8.3 the slope of the lines which connect points in the surface of the model at the same total acid concentration, the experimental data can be supplemented; thus sections through the model at these pH values may be established with sufficient accuracy for present purposes.

This second model now corrects a possible misconception gained from the first. Both models show that at pH 8.3 (where free carbonic acid can be neglected) the depressing effect of added bicarbonate (total carbonic acid present in the system) on bromide uptake is great. This effect must be due either to $[HCO_3^-]$ or to this supplemented by $[OH^-]$ since the only free undissociated acid is that which arises from hydrolysis. The effect of increased concentration upon bromide absorption, *per unit of total carbonic acid present*, at *constant pH*, is a maximum at pH 8.3. It decreases progressively at more acid reactions at which relatively more of the acid present is in the uncombined and undissociated form. This must mean that, as between bicarbonate ion and undissociated free acid, the *most effective component of the bicarbonate buffer system which suppresses the uptake of bromide is the bicarbonate ion itself*. It is equally clear, however, that other factors are involved. Were it not so, all of the data could be fitted to a smooth curve of bromide uptake plotted against bicarbonate ion concentration and this is not possible. Solutions which are acid due to carbon

[2] Where such sections cut the surface of the model is shown by a chain-dotted line in the figure (.–.–.–).

[3] Where such sections cut the surface of the model is shown by a broken line in the figure (– – – – – – – –).

dioxide, unbuffered by potassium bicarbonate, exert on bromide uptake a retarding effect which is out of all proportion to the bicarbonate ion which they contain; this suggests that the free carbonic acid also has its specific effects on the bromide absorbed—either due to the undissociated free acid or the H^+ *per se*.

In the absence of added bicarbonate the bromide uptake is but little affected by increased concentration of dissolved carbon dioxide between pH 7.7 and 5.9; but at more acid reactions it is depressed and, in the pH range 7.7 to 4.7, the data fall on a straight line, the empirical equation of which is $[Br] = 17.3 - \frac{17.3}{17.0} \times [\text{dissolved } CO_2]$ where the concentrations of bromide in the sap and carbon dioxide in the solution are expressed in milligram equivalents and millimols per liter, respectively.

In the presence of added bicarbonate the effect of increased carbon dioxide on bromide accumulation is due to the combined action of the bicarbonate and the free acid. Although the concentration of salt and free acid is either known or can be calculated at the given pH values, the data are hardly adequate to derive a satisfactory empirical relationship between dissolved carbon dioxide, bicarbonate, pH or $[H^+]$, and bromide absorption which covers the full range of conditions. Such a relationship could not do more than describe concisely the models shown—it would not alone explain the effects of bicarbonate and carbon dioxide on bromide uptake, since these factors clearly operate through the metabolic processes which are involved in absorption and which have yet to be described.

It happens that all of the data in tables I and II lie on a smooth curve of bromide uptake plotted against total carbonic acid in the external solution. This relation is not general, however, and would probably not hold at high concentrations of total carbonic acid and reactions more alkaline than pH 7.7.

At any given pH value, a simultaneous increase of potassium bicarbonate and dissolved carbon dioxide retarded, and eventually suppressed, bromide uptake. Both salt (bicarbonate ion) and free undissociated acid (H_2CO_3) contributed to this effect which was clearly not due to the H and OH ions. If the effect of bicarbonate is additive to that of carbonic acid, then simple calculations show that at constant pH, near neutrality, the drop in bromide concentration in the sap due to the external bicarbonate was 1.1 milligram equivalents per liter of sap per milligram equivalent of bicarbonate in 1 liter of external solution.

The retardation of bromide uptake which was due to increase of potassium bicarbonate concentration at constant pH is the more interesting because increased concentrations of other potassium salts (with anions Cl, Br, NO_3) stimulate all those processes which are concerned in salt accumu-

lation (**22**). Therefore, the processes which are deemed essential to salt uptake should be suppressed by increased concentrations of total carbonic acid at constant pH and evidence to be described shows that this is, in fact, the case.

SPECIFIC EFFECTS OF pH ON BROMIDE UPTAKE.—An obvious difficulty is to ascertain from the evidence whether H^+ and OH^- have any direct effect upon bromide uptake. In solutions enriched with bicarbonate, the bromide uptake falls off more at alkaline reactions than near neutrality and this result is evident from the models (figs. 3 and 4) whether one compares cultures of constant potassium bicarbonate concentration or of the same *total* carbonic acid concentration. If the bromide uptake was influenced only by the bicarbonate ion and the free acid then, since their relative effects are almost equal at pH 7.0, the effect of increased carbonic acid in the system should be independent of pH provided the *total* (bicarbonate and dissolved carbon dioxide) remained constant. Figure 4 shows that such is not the case. At reactions more acid than pH 5.9 and more alkaline than 7.2, bromide uptake is less than at the intermediate reactions; this effect becomes the more conspicuous as the total capacity of the tissue to absorb is retarded by carbon dioxide and bicarbonate (see vertical section through model of figure 4 along 0.014×10^{-3} mols total CO_2 per liter). Such effects obtained in the more acid and more alkaline reactions *are attributable only to acidity and alkalinity per se* and, therefore, to specific effects of H^+ and OH^-. This statement is made only after a full attempt to account for the effects observed *solely* on the basis of the calculated concentrations of bicarbonate ion and undissociated carbonic acid without reference to H^+ and OH^-.

There is thus *a range, relatively broad in the absence of bicarbonate, within which bromide uptake is not much affected by hydrogen ion concentration and its attendant variables. Reactions near to neutrality, however, are at any given total concentration of added carbonic acid, more favorable to bromide uptake than either more acid or more alkaline solutions.* The investigation of the effects of carbonic acid and bicarbonates on metabolism shows that metabolic processes now known to be closely associated with bromide uptake are similarly affected and show optima near pH 7.0. The view that these are, in fact, effects due to H and OH ions is strengthened because similar results have been obtained in solutions of other buffer salts; *e.g.*, phosphates.

These data predict the acid reactions at which bromide uptake should vanish. In solutions unbuffered by bicarbonate there are no data above pH 8.3. The form of the models suggests that at still more alkaline reactions bromide uptake might also decline and eventually vanish. This problem, upon which it is interesting to speculate, can be solved only if experiments are carried out with other buffer systems, or in a range of pH where the second dissociation of carbonic acid operates.

TABLE II

MOLS OF ADDED BICARBONATE AND TOTAL ACID (COMBINED AND UNCOMBINED H_2CO_3) IN SOLUTIONS USED

pH	Added $KHCO_3$	Total acid	pH	Added $KHCO_3$	Total acid	pH	Added $KHCO_3$	Total acid	pH	Added $KHCO_3$	Total acid
	mols.	*mols.*		*mols.*	*mols.*		*mols.*	*mols.*		*mols.*	*mols.*
4.75	–	0.00940	5.3	0.001	0.01400	7.23	0.005	0.00574	6.81	0.0025	0.00349
5.92	–	0.00270	7.3	0.001	0.00113	7.23	0.010	0.01148	6.76	0.0150	0.02150
6.12	–	0.00149	8.3	0.001	0.00101	.....	.....	.....	6.81	0.0200	0.02780
6.58	–	0.00028	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE III

EFFECT OF INCREASED [$KHCO_3$] AND [CO_2] AT pH 6.5 ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. AND 72 HOURS*

Sample	[$KHCO_3$] per liter	Protein N per gram fresh weight	Soluble N per gram fresh weight	Protein N	Soluble N	Amino N	Amide N		Ammonia* N
							Stable	Unstable	
	equiv.	*mg.*	*mg.*	%	%	%	%	%	
Initial	–	0.58	1.44	28.7	71.3	56.5	8.4	7.4	.....
Final	0.005	0.90	1.16	43.7	56.3	45.6	4.9	5.8	.....
"	0.020	0.65	1.35	32.5	67.5	51.0	6.0	12.0	.....
"	0.040	0.65	1.40	31.7	68.3	50.8	6.3	10.7	.....

* Absolute units milligrams nitrogen per gram initial fresh weight. Results on a percentage basis are relative to total nitrogen. Negligible—0.001 mg. per gm. fresh weight.

SUMMARY OF THE EFFECTS OF THE COMPONENTS OF THE HCO_3^-/H_2CO_3 BUFFER SYSTEM ON BROMIDE UPTAKE.—The effect of bicarbonate and dissolved carbon dioxide on bromide uptake is complex. The components of the carbon dioxide buffer system *depress absorption*. Combined and free carbonic acid both depress bromide uptake and at pH 7.0 their relative effects are approximately equal. The indications are that the undissociated free acid in the system is less effective than the bicarbonate ion in retarding bromide uptake. A full interpretation of all the facts cannot evade effects which must be ascribed specifically to H and OH ions—effects which operate in such a fashion that reactions near neutrality become especially conducive to bromide uptake. This is particularly true when the total absorption is already reduced by the effects of added carbonic acid.

THE EFFECTS OF pH, DISSOLVED CARBON DIOXIDE, AND POTASSIUM BICARBONATE ON THE METABOLISM OF POTATO DISCS

THE EFFECT OF INCREASED $KHCO_3$ AND CO_2 CONCENTRATION AT pH 6.5.—The most striking feature of the effect of the components of the CO_2/HCO_3^- buffer system upon bromide uptake is that at a constant pH, bromide accumulation is progressively decreased, and eventually suppressed, by the simultaneous increase of potassium bicarbonate and dissolved carbon dioxide. This is true even though all other variables remain at values conducive to a high degree of salt accumulation. If bromide absorption is to be correlated with a particular aspect of metabolism, then this should be a process which is retarded by these treatments. Therefore, investigation of the metabolic processes so affected will go far to identify those which are essentially concerned in bromide absorption.

Discs exposed to aerated solutions of potassium bicarbonate at constant pH do not brown as much in the strong as they do in the dilute solutions or in distilled water. Usually (**21, 22**) increased concentrations of potassium salts tend to accentuate the surface browning reaction. Since bicarbonates are unusual in this respect, it is to be expected that the specific effects of the bicarbonate and dissolved carbon dioxide on metabolism would operate through processes which are linked with the activity of the oxidase (phenolase) system of the potato tuber. Experiments which were carried out at pH 6.5, show that this is the case; this is the pH at which the buffer effect is at its maximum.

From table III and figure 5, it is apparent that the protein synthesis which normally occurs in potato discs in distilled water or in dilute potassium bicarbonate solution is *almost completely suppressed* by an external concentration of 0.02 mols per liter and its attendant carbon dioxide concentration. This result, as well as much other evidence not given here (**21, 22, 23**) suggests that protein synthesis plays an indispensable part in the accumulation of bromide by potato discs.

Further analysis of the soluble nitrogen fractions shows that, as previously recorded (**21, 22**), the nitrogen used in synthesis is mainly amino-N.[4] The treatments which in the experiment of table III depressed synthesis (compare cultures at 0.005 and 0.020 equiv. $KHCO_3$) also retarded the utilization of amino-acid but *not to the same degree.* When there was little gain of protein in the tissue (cultures at 0.020 and 0.040 equiv. $KHCO_3$) more amino acid disappeared than reappeared as protein and the excess was accounted for by an increase in the unstable amide-like compound which has previously been noted (**21**) and which was then regarded as a possible reactive intermediary between amino-acid and protein. It thus appears that the combined effect of bicarbonate and carbon dioxide is not exerted exclusively upon the deamination of the amino-acids, but even more upon the

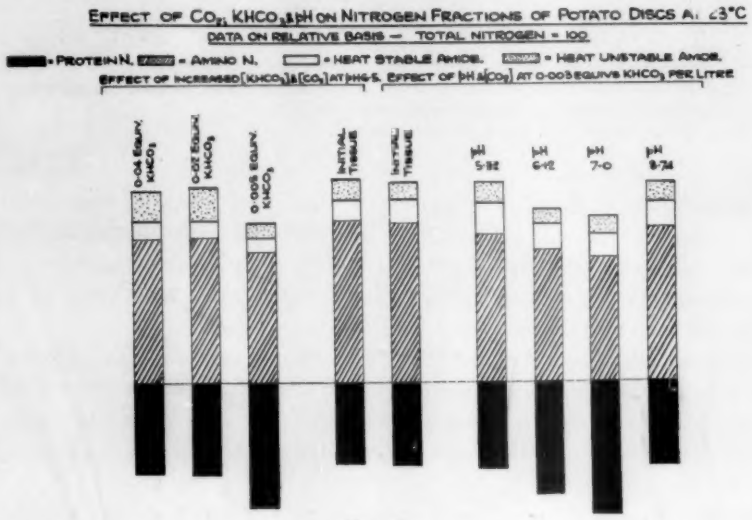
EFFECT OF CO_2, $KHCO_3$ & pH ON NITROGEN FRACTIONS OF POTATO DISCS AT 23°C
DATA ON RELATIVE BASIS — TOTAL NITROGEN = 100
= PROTEIN N. = AMINO N. = HEAT STABLE AMIDE. = HEAT UNSTABLE AMIDE.
EFFECT OF INCREASED [$KHCO_3$] & [CO_2] AT pH 6·5. EFFECT OF pH AT [CO_2] AT 0·005 EQUIVS $KHCO_3$ PER LITRE
0·04 EQUIV. $KHCO_3$
0·02 EQUIV. $KHCO_3$
0·005 EQUIV. $KHCO_3$
INITIAL TISSUE
INITIAL TISSUE
pH 5·92
pH 6·42
pH 7·0
pH 8·74

FIG. 5.

later stages of protein synthesis in which the reactive intermediaries are converted to protein. As the intermediates accumulate in bicarbonate cultures which depress synthesis they, in turn, tend to depress the deamination of the amino-acids. It will be recalled that when potassium salts stimulate synthesis they do so by affecting both the deamination of amino acid and the use of the reactive amides which are supposed to be intermediates in synthesis. When calcium salts depress synthesis, however, the unstable amides do not accumulate.

THE EFFECT OF pH AT CONSTANT BICARBONATE CONCENTRATION.—Superimposed upon the effect of bicarbonate and dissolved carbon dioxide, which at constant pH does not concern the H and OH ions, there is at any given

[4] Amino-N free from confusion with heat unstable amides which react both in the amide and the VAN SLYKE determination.

concentration of $KHCO_3$, an effect on protein synthesis which must be due primarily to these ions. This is shown by the data of table IV which are

TABLE IV

EFFECT OF pH IN $KHCO_3$ BUFFERED SOLUTIONS (0.003 EQUIV. PER LITER) ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. AND 72 HOURS*

SAMPLE	pH	PROTEIN N PER GM. FRESH WT.	SOLUBLE N PER GM. FRESH WT.	PROTEIN N	SOLUBLE N	AMINO N	AMIDE N		AMMONIA† N
							STABLE	UNSTABLE	
		mg.	*mg.*	%	%	%	%	%	
Initial	.....	0.62	1.46	29.8	70.2	55.0	8.6	6.2	.....
Final	5.32	0.63	1.42	30.7	69.3	51.7	11.2	6.3	.....
"	6.12	0.82	1.25	39.6	60.4	46.3	9.2	4.9	.....
"	7.00	0.95	1.09	46.6	53.4	43.6	8.4	6.4	.....
"	8.74	0.59	1.39	29.8	70.2	54.0	9.0	6.5	.....

* Absolute units, milligrams nitrogen per gram initial fresh weight. Results expressed on a percentage basis are relative to total nitrogen.

† Negligible—0.001 mg. per gm. fresh weight.

illustrated in figure 6. It is clear that *protein synthesis is at a maximum at pH 7.0* and that *it declines both in more acid and more alkaline solutions.* It will appear later that similar results are obtained in phosphate buffered solutions although specific effects of phosphate and bicarbonate on protein synthesis are quite different. This effect of pH must, therefore, be due to H and OH ions specifically.

The detailed analyses of the soluble nitrogen fractions simply show that the effects of pH are exerted mainly upon the utilization of the amino-acid fraction. The changes in total soluble nitrogen and amino nitrogen run parallel throughout and they are complementary to the observed change in protein nitrogen (table V).

Combining now the results at constant pH and at constant bicarbonate concentration in figure 6, it is impossible to ignore the similarity between the effects of these treatments on protein synthesis and on bromide accumulation. In both cases the process in question is retarded and eventually suppressed by increased concentrations of bicarbonate and dissolved carbon dioxide; at a given salt concentration, it exhibits an optimum at a pH value of 7.0. It is to be concluded, therefore, that the specific effects of the components of the H_2CO_3/HCO_3^- buffer system and of pH on bromide absorption are linked with their effect on protein synthesis—a process with which bromide uptake in potato discs is closely associated.

EFFECT OF pH, BICARBONATE, AND DISSOLVED CARBON DIOXIDE ON THE RELATIVE ABSORPTION OF ANION AND CATION

The absorption of potassium in the experiments of table I presented a

TABLE V

EFFECT OF pH, [$KHCO_3$] AND [CO_2] ON ACCUMULATION OF POTASSIUM BY POTATO DISCS AT 23° C. DURING 70 HOURS

EXP. NO.	[K] IN EXTERNAL SOLUTION	[$KHCO_3$] IN EXTERNAL SOLUTION	MEAN pH	GAIN OF K IN SAP	EXP. NO.	[K] IN EXTERNAL SOLUTION	[$KHCO_3$] IN EXTERNAL SOLUTION	MEAN pH	GAIN OF K IN SAP	K ABSORBED IN EXCESS OF Br
	equiv./l.	*equiv./l.*		*mg. equiv./l.*		*equiv./l.*	*equiv./l.*		*mg. equiv./l.*	*mg. equiv./l.*
1	0.001	.....	4.75	− 9.6	2	0.011	0.001	7.30	11.0	.....
2	0.011	.....	5.92	− 5.7	2	0.011	0.005	7.23	20.0	8.5
2	0.011	.....	6.12	+ 0.2	2	0.011	0.010	7.23	14.5	7.7
2	0.011	.....	6.58	+ 5.5	3	0.021	0.025	6.81	19.1	5.0
1	0.001	.....	6.90	+17.4	3	0.021	0.015	6.76	32.9	30.0
3	0.021	.....	7.70	+ 9.4	3	0.021	0.020	6.81	22.5	21.0

more difficult problem than that of bromide. It is complicated by the effect of the treatment on the high concentration of potassium which existed in the initial tissue (76 mg. equiv. per liter of sap) and which, under conditions unfavorable for absorption, may leave the cells. It is necessary to examine the results obtained to see whether these variables affect potassium and bromide absorption in a similar fashion and to ascertain whether the evident connection between protein synthesis and bromide uptake applies equally to potassium absorption.

The effect of pH on the absorption of potassium and on the potassium content of the initial tissue can be seen in the first part of table V.[5] The different experiments were conducted at various total potassium concentrations which are shown, though they were all at constant bromide concentration and in the absence of bicarbonate. It is clear that the concentration of the only mobile anion (Br), which was constant, had a greater control over the uptake of cations than the varying concentration of potassium sulphate in the range shown. If potassium sulphate concentration had any tendency to increase potassium absorption, allowance for this would merely accentuate the effect of pH to which reference will now be made.

At neutrality (strictly pH = 6.9) absorption of potassium and bromide was in equivalent amounts—a fact which has often been observed for potato discs in potassium bromide solutions. At more acid reactions, due to increased concentrations of carbon dioxide, there was an apparent decrease in the potassium absorbed. This was due to the effect on the absorption of potassium bromide but also to the effect of carbon dioxide on the loss of potassium which was present initially in the tissue. This loss was so great at acid reactions (pH $<$ 6.0) that it entirely masked the potassium absorbed along with bromide. Although the data are limited at reactions more alkaline than pH 7.0, there is a strong suggestion that these also caused loss of the potassium previously stored in the cells. The obvious conclusion is that the tissue retains its stored potassium best under those conditions most suitable for protein synthesis.

Reference may be made here to a similar, though even more striking, case to which a similar conclusion applies. After prolonged storage of tubers at low temperatures, potato discs no longer retain their salts against aerated distilled water (**17**, p. 536); they lose the capacity to grow as shown by meristem formation in moist air, and *it is now known that they also lose their ability to synthesize protein.*[6] Though as yet unpublished, these results strengthen the conviction that the observed effect of pH and carbon dioxide concentration on the loss of potassium from the cells is causally connected with their effect on protein synthesis (fig. 6). This suggests that,

[5] For these potassium analyses we have to thank members of the laboratory of Plant Nutrition, University of California, Berkeley, California.

[6] Data in thesis of T. K. RAMAMURTI, University of London.

in order to retain their solutes against distilled water, the cells cannot merely remain static; they must also be capable of synthesizing protein. The evident connection between protein synthesis and the ability of cells to retain potassium in their sap, recalls previous observations that the discs must maintain an unexpectedly high rate of respiration in order to retain

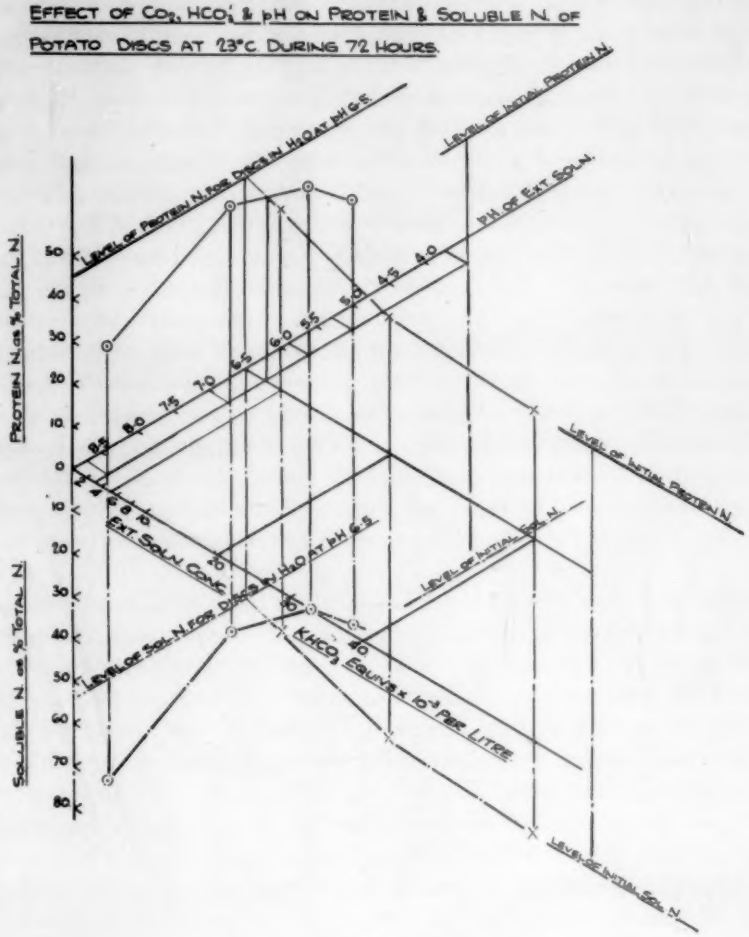
EFFECT OF CO_2, HCO_3^- & pH ON PROTEIN & SOLUBLE N. OF POTATO DISCS AT 23°C DURING 72 HOURS.
LEVEL OF PROTEIN N. FOR DISCS IN H_2O AT pH 6.5
LEVEL OF INITIAL PROTEIN N.
pH OF EXT SOLN.
PROTEIN N. as % TOTAL N.
SOLUBLE N. as % TOTAL N.
EXT SOLN CONC.
LEVEL OF SOL. N. FOR DISCS IN H_2O AT pH 6.5
LEVEL OF INITIAL SOL. N.
$KHCO_3$ EQUIVS. $\times 10^{-3}$ PER LITRE
LEVEL OF INITIAL SOL. N.

FIG. 6.

their salts (**16**, pp. 215–234). These results stand in contrast to the long-cherished conception of a passive "semi-permeable" structure by which cells retain the solutes contained in their vacuoles. It must be recognized that the metabolic processes of respiration and protein synthesis are equally necessary to retain salts *after they are accumulated* as they are to produce the high internal salt concentration *de novo*.

The effect of bicarbonate and dissolved carbon dioxide concentration at constant pH is revealed by two series of data from experiments at different total potassium concentrations. In the presence of added bicarbonate, the absorption of potassium always greatly exceeded the absorption of bromide, even at reactions close to neutrality (pH 7.23 and 6.8). At contant pH, the potassium absorbed in excess of bromide increased with bicarbonate concentration up to an optimum concentration above which, presumably owing to the effects of dissolved carbon dioxide, further increase reduced the total absorption of potassium as well as its excess over the bromide absorbed. There can be little doubt that the *potassium absorbed which was unaccompanied by bromide was absorbed along with bicarbonate ion* and that this process occurred under conditions such that *protein synthesis and bromide uptake were reduced almost to zero* (culture at 0.020 mol. $KHCO_3$ and pH 6.8, table V). The absorbed bicarbonate, however, did not remain as such in the sap; when this was acidified, it yielded little more carbon dioxide than did the normal sap. The presumption is that potassium bicarbonate was absorbed as such and reacted with the organic acids of the sap. This process represents at least a transitory means by *which the cells accumulate potassium independently of protein synthesis and of growth* although it can persist only so long as the stored reserves of organic acids remain or are replenished. The final result would be the same if potassium exchanged for hydrogen ion supplied by the organic acid. Some may prefer this interpretation but it leaves the rôle of bicarbonate in the solution without explanation.

Further work on this point is necessary but there seem to be grounds for homologizing this uptake of potassium by potato discs with the brief but rapid uptake of potassium by "low salt" barley roots during which, if it occurs from potassium bicarbonate, organic acids increase in the cell sap. Potassium may be absorbed, therefore, by distinct mechanisms which bear different relationships to metabolism. The absorption of potassium from bicarbonate solutions presents a very special case—it is clearly less dependent upon certain aspects of metabolism (protein synthesis and processes linked with it) than the absorption of potassium bromide. The extent to which it is conditioned by oxygen in the external solution remains an interesting problem for the future.[7]

The unequal absorption of anion and cation may be a contributory cause of the metabolic effects which obtain in bicarbonate solutions. HOAGLAND'S observation that barley roots respond to bicarbonate absorption by actual increase in their organic acid content has been noted. Indications that the centers of protein synthesis in potato discs respond to unequal absorption

[7] In barley root experiments, HOAGLAND and BROYER have shown that the absorption of potassium from bicarbonate solutions does not occur at very low oxygen concentrations (private communication to the authors).

of cation and anion as though they operate best under conditions of strict neutrality, have been noted elsewhere (**21**). Equal uptake of both ions from some potassium salts (KBr) with its concomitant synthesis and the fact that both synthesis and uptake are increased by greater concentrations, contrast with the unequal uptake of calcium salts (accentuated perhaps by the possible fixation of the cation as insoluble compounds) and the depression of synthesis which greater concentrations cause. In potassium bicarbonate solutions the final effect is as if the cation were absorbed unaccompanied by anion (or exchanged for hydrion) and this condition of ionic unbalance is associated with a markedly depressed protein synthesis. Moreover, both in phosphate and bicarbonate buffers at constant total acid the synthesis is a maximum at a neutral external reaction of 7.0. Hence the relation between protein synthesis and salt uptake is not merely that synthesis is a vital property of growing cells which makes uptake possible. It is that continued absorption, like synthesis, does not tolerate the consequences of ionic unbalance due to unequal intake of anions and cations by the cells and it is favored by external solutions with reactions close to strict neutrality. If disturbance in the metabolic machinery is the consequence of such ionic inequality, one may well look for its explanation in the behavior of the organic and amino acids. The whole tendency is to conserve the latter in the presence of bicarbonate and avert their conversion to protein.

The effect of pH, potassium bicarbonate, and carbon dioxide concentration on the respiration and metabolism of carbohydrate of potato discs

The direct determination, by carbon dioxide evolved, of the respiration of potato discs in solutions rich in bicarbonate and dissolved carbon dioxide presented too great technical difficulties to be profitable. Indirect determinations by the change in total carbon in the tissue due to respiration was complicated by the loss of carbon which the tissue sustains when it is blotted dry with paper (**23**) and by the absorption of bicarbonate from the external solution. Consequently, the effect of the treatments already described on respiration cannot be stated very precisely. It is clear, however, that respiration, like the phenolase activity and protein synthesis, was retarded at pH 7.0 in the presence of bicarbonate (0.020 N) and dissolved carbon dioxide (solution in equilibrium with 20 per cent. CO_2 by volume). This was demonstrated by using tissue of a variety different from that used in experiments 1, 2, and 3, and by comparing the total heat content (bomb calorimeter determination), starch and sugar content of initial washed discs, and comparable samples after 70 hours of contact with either bicarbonate solution at pH 6.5 or the equivalent strength of potassium sulphate.

In the tissue in the bicarbonate solution the hydrolysis of starch to sugar (final sugar, 0.24 gm.; final starch, 3.10 gm. per 40 gm. initial fresh weight) was retarded in comparison with that which received the sulphate treatment (final sugar, 0.34 gm.; final starch, 2.75 gm. per 40 gm. initial fresh weight). The total carbohydrate recovered (starch + starch equivalent of final sugar) was greater in the bicarbonate culture than in the sulphate. The still outstanding loss of carbohydrate (initial tissue contained 4.16 gm. and the bicarbonate treated 3.32 gm. total starch per 40 gm. fresh wt.) in the tissue treated with bicarbonate solution at pH 6.5 was due partly to carbon losses incurred in blotting the discs (**23**), and partly to respiration or other metabolic processes which were not separately measured. One must make the same allowance (estimated at 0.42 gm. per 40 gm. initial tissue) for loss of carbohydrate owing to the formation of the surface film of mucilage in the bicarbonate series, as that which is necessary to "balance" the carbohydrate balance sheet of the potassium sulphate series. It then appears that at pH 7.0 in contact with 0.020 N potassium bicarbonate and the appropriate carbon dioxide concentration, 40 gm. of this tissue (in which protein synthesis was depressed but not completely eliminated) respired 0.42 gm. of starch in 70 hours as against 0.64 gm. in bicarbonate and carbon dioxide-free solutions. The total heat change in the discs also indicated that respiration was depressed in presence of bicarbonate and dissolved carbon dioxide but the precise effect must be determined by other methods. Knowing the general parallelism between the effects of other salts on respiration and protein synthesis (**23**) and also the phosphate experiments in this paper, the trend of the effect of the bicarbonate and carbon dioxide treatments on respiration may be inferred from their effects on protein synthesis which are here recorded. Adopting this standpoint, the now oft-repeated parallelism between bromide absorption and respiration of potato discs would again emerge—a treatment (increased bicarbonate concentration) which depresses bromide uptake has a similar effect on respiration.[8]

Carbon dioxide has somewhat unexpected results on living cells. The observations of THORNTON (**25**) and FIFE and FRAMPTON (**3**) have given prominence to an effect of carbon dioxide on the pH of the cell and show that this is not always predictable on *a priori* grounds. FIFE and FRAMPTON showed that the explanation of the unexpected shift toward alkalinity in the sap of carbon dioxide-treated cells lies in its effect on systems in the cells which catalyze the hydrolysis and re-synthesis of amides—reactions which occur only in the living tissue in the presence of oxygen (**3, 25**). Actual tests showed that this effect did not enter appreciably into the behavior of

[8] Evidence from the researches of A. ULRICH on barley roots (in course of publication) suggest that the respiratory quotient may be altered when accumulation of K occurs from a solution of $KHCO_3$. Further investigation of this possibility with reference to potato tissue would be of interest.

immersed potato discs at pH 7.0. FIFE, at the author's request, made tests in 1934 which showed that the shift in pH toward alkalinity in the sap expressed from potato discs which have been exposed to high concentrations of dissolved carbon dioxide (equilibrium with 2 atmospheres pressure CO_2) was only slight (pH 0.26). At the same time, the detailed analysis in tables III and IV did not show the free ammonia to be expected if amide hydrolysis had occurred extensively. It will be recalled, however, that the tissue at pH 7.0 in solutions rich in bicarbonate and dissolved carbon dioxide did not show the usual symptoms of oxidase activity and it is here, through some esential property of the living protoplast, that the mechanism must be sought. From the evidence presented it is clear that the effect of bicarbonate and dissolved carbon dioxide on metabolism embraces the nitrogen compounds although mainly in a way other than that which FIFE and FRAMPTON (3) described for the beet plant exposed to carbon dioxide gas. The response of beet leaves to CO_2 is rapid and considerable (increases of pH approaching 1.0 pH unit were observed by FIFE and FRAMPTON in 60 minutes). THORNTON's work was done on much larger masses of tissue in a gas phase and the response, though considerable, required a longer period. A possibility that thin discs would react even more than the large masses was evidently not realized. This difference is most probably due to factors incidental to the use of discs immersed in aerated solution, though the possibility that the reversibility of the effect, which FIFE and FRAMPTON observed for beets, is so accentuated in these discs that their reaction is reversed before sap can be expressed and measured may have to be considered. Even if this were the case, the clue to the metabolic effect of carbonic acid and bicarbonate solutions should be with the permanent, rather than the transient, results of such treatments.

Experiments made on the effect of pH and phosphate concentration on the respiration and nitrogen metabolism of potato discs afford an interesting comparison with the bicarbonate series from which the effects specifically due to H and OH ions may be inferred.

THE EFFECT OF pH AND PHOSPHATE CONCENTRATION ON THE METABOLISM OF POTATO DISCS

The trend of the effects of total phosphate concentration and of pH in phosphate buffered solutions is shown by the results of two series of experiments. One is conducted at constant pH and the other at constant total concentration of total phosphate and potassium; otherwise both are conducted under identical conditions of time, temperature, aeration, etc. These variables are fixed at the same arbitrary values used in the bicarbonate experiments so that direct comparisons can be made between the two series of experiments.

The effects due to varying concentration of total phosphate were determined at pH 6.9. This is a reaction close to strict neutrality and at the pKa value of phosphoric as a dibasic acid where the buffering effect of potassium dihydrogen phosphate in presence of potassium monohydrogen phosphate is at its maximum. The effects due to pH were determined at constant concentration of 0.020 mol per liter of total phosphate. To eliminate confusion due to variable concentration of potassium, the solutions had their potassium content raised where necessary, to a constant level of 0.040 equivalent per liter, by the addition of potassium since the sulphate ion has only slight influence on the metabolism of potato discs. The composition of the solutions used is given in table VI.

TABLE VI

COMPOSITION OF BUFFER MIXTURES FOR EXPERIMENTS AT CONSTANT PHOSPHATE AND POTASSIUM CONCENTRATION*

pH	SOLUTION A TO 2 LITERS	SOLUTION B TO 2 LITERS	SOLUTION C TO 2 LITERS
	ml.	*ml.*	*ml.*
8.04	133	137.0	.....
7.46	133	112.0	25.0
6.95	133	73.0	64.0
6.48	133	33.0	104.0
5.96	133	14.6	122.4
6.82	.....	.....	270.0

* Stock Soln. A = 0.3 M KH_2PO_4.
Stock Soln. B = 0.3 M KOH.
Stock Soln. C = 0.15 M K_2SO_4.

To arrive at these desired mixtures stock solutions of 0.3 M KH_2PO_4 and KOH were prepared and, using the glass electrode, the titration curve of the one against the other was determined and from this the volume mixtures necessary for any desired pH could be ascertained.[9]

For the experiment at constant pH a phosphate buffer mixture at pH 6.9[10] was prepared which was then diluted so that the desired total phosphate concentrations were obtained; namely, 0.002 M, 0.01 M, 0.025 M, and 0.040 M. The last was too strong and, because of the less turgid condition of the tissue in this solution, no safe conclusions could be drawn from this culture. These data are therefore omitted.

The effect of the above treatments can be represented by a method similar to that previously adopted for the bicarbonate experiments. Respiration rate and protein, or soluble nitrogen, content of the discs were plotted on isometric paper against *both* pH of the external solution *and* the

[9] See HOLT, LAMER, and CHOWN (7) for a discussion of the ionic equilibria in phosphate solutions at different pH's; also CLARK (2).

[10] One liter of 2 M/5 KH_2PO_4 + 720 ml. 2 M/5 KOH, the mixture diluted to 2 liters.

total phosphate concentration. The data obtained identify two sections cut through a solid model, the surface of which describes the inter-relationships of the three variables concerned. Sufficient data to identify such surfaces completely would involve a rather formidable investigation which it might be difficult to complete before the changes which ensue during storage affected the behavior of the tissue. The experiments were so planned, however, that the more limited data available permit one to discern the general trend in the effects which were obtained.

RESPIRATION.—The time curve of respiration of tissue in phosphate buffered solutions at pH 6.9 is identical in form to that already shown for tissue in distilled water or certain neutral salt solutions ($CaCl_2$). It consists of an initial period during which the respiration rises to a value which is subsequently maintained for long periods. Phosphate cultures at acid or alkaline reactions introduced further complications into the initial period and it is not proposed to deal with these at length here. It will suffice to state that the carbon dioxide removed by the air stream from the cultures in the first period increased progressively from the more alkaline to the more acid solution. This was doubtless due to the bicarbonate which remained in the more alkaline solution. Later in the time drift cultures, both more acid and more alkaline than pH 6.9, it produced a transient increase in respiration; this had, however, elapsed before the period of 26 to 72 hours to which particular attention will be directed.

The effect of increased concentration of total potassium phosphate on respiration is revealed by the series at constant pH (table VII). Those

TABLE VII

EFFECT OF EXTERNAL pH ON RESPIRATION* OF POTATO DISCS IN POTASSIUM PHOSPHATE BUFFERS (0.020 M) OF CONSTANT K CONTENT (0.040 EQUIV.) AT 23° C.

External pH	5.96	6.48	6.95	7.46	8.04
Respiration rate	0.207	0.299	0.325	0.302	0.234
Relative respiration rate	63.8	92.0	100.0	93.0	72.0

* Respiration rates = mean rates in mg. CO_2 per gm. initial fresh wt. per hour for period 26 to 72 hours of exp.
Relative respiration rate = culture at pH 6.95 as 100.

effects are due either to potassium or to the simultaneous and proportional increase in $H_2PO_4^-$ and HPO_4^{--}; the relative amounts of these are equal in solutions of pH equal to the pKa value of the second dissociation of phosphoric acid (pH = 6.9). Total phosphate concentration increased respiration at pH = 6.9 (table VIII, fig. 7); this might be expected from the prevalent belief that hexose phosphate plays a prominent rôle in respiration.

It is well to emphasize, however, that the increase observed is not conspicuously greater than the response obtainable with potassium nitrate and

TABLE VIII

EFFECT OF TOTAL CONCENTRATION OF POTASSIUM PHOSPHATE BUFFER ON RESPIRATION RATE* OF POTATO DISCS AT pH 6.9 AND 23° C.

Molar concentration phosphate	0.0	0.002	0.010	0.025
Respiration rate	0.193	0.204	0.245	0.367
Relative respiration rate†	100.0	106.0	127.0	190.0

* Respiration rates = mean rates in mg. CO_2 per gm. initial fresh wt. per hour for period 26 to 72 hours of exp.

† Relative respiration rate = culture in distilled water = 100.

it is actually less than the response which has been obtained in ammonium sulphate and nitrate solutions (unpublished experiments). The interpretation of the mechanism of the phosphate response can be deferred, but it is clear that the doubtful specific effect of phosphate, unlike that of bicarbonate, is to increase respiration.

Despite the contrast in the effects due to the two anions, the effects of pH on respiration in phosphate buffered solutions (table VII, fig. 7) are similar to those of pH on protein synthesis (and presumably also on respiration) in bicarbonate buffered solution. After the respiration had attained somewhat steady levels during the last 36 hours of the treatments, the maximum respiration occurred at pH 6.9. When data from the two experiments are combined (fig. 7) the probable form of the surface which depicts the effect of phosphate concentration and pH on the respiration of potato discs can be visualized.

THE GENERAL EFFECT OF pH ON RESPIRATION.—The literature contains no other record of the effect of phosphate concentration and pH on the respiration of potato discs similar to figure 7. Reported attempts to show the effect of pH suffer from inadequate appreciation of the variables which affect the behavior of potato discs. LEMMON (**8**) and BOSWELL (**1**), both working with potato tissue, used the WARBURG manometric method—a technique the full implications of which have not been adequately investigated relative to the behavior of potato discs. LEMMON was primarily concerned with the effects of pH on respiration but used buffer solutions of such varied composition that the effect of pH *per se* could not be segregated from that due to other variable components of the solutions. This was doubtless responsible for the erratic behavior observed.

BOSWELL though not primarily concerned with the effects of either salts or pH on respiration, used such short time periods (0 to 3 hours) that it is questionable if the tissue had fully responded either to the salt treatment or to the oxygen content of the solution if this was indeed in equilibrium with air. BOSWELL did not state the full composition of his buffer solutions; *e.g.*, the nature and concentration of the cation. He remarks that his results

show that for the pH range of 5.29 to 8.04, the oxygen uptake is unaffected between pH 5.59 and 6.81 "falls away slightly below 5.9 and rises above 6.81." No final conclusion can be drawn from his figures which merely show that in his experiments the effect of pH, if indeed pH was the causal factor, was erratic. The pH effects described in this paper, whatever their ultimate explanation, are clearly consistent with the other results of this

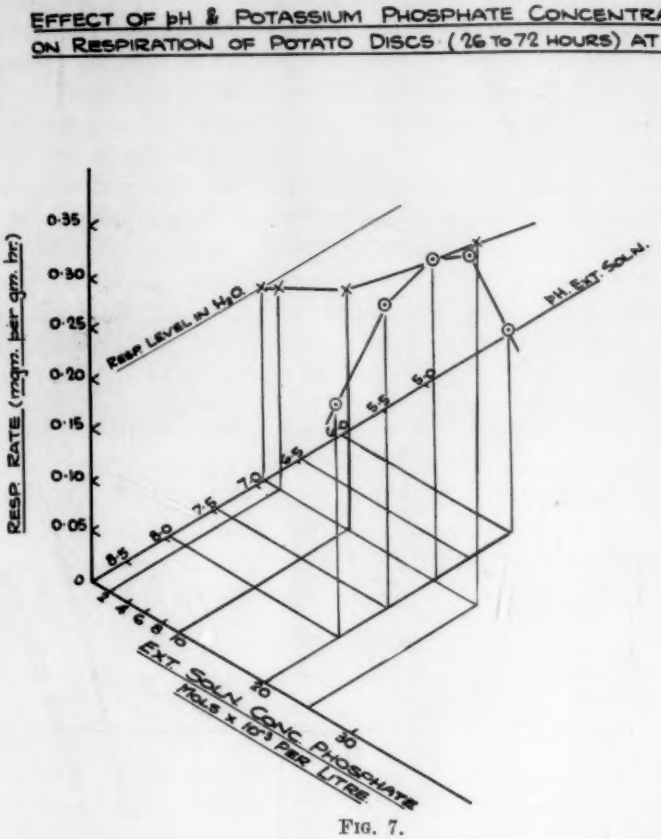

FIG. 7.

series of investigations and they form part of a comprehensive, general picture of the behavior of potato tissue.

With respect to other tissues there are inconsistencies in the relation of respiration to pH. To cite but two cases: LYON's (10) results with Elodea showed greater respiration with increasing alkalinity beyond pH 7.0; whereas THOMAS (24) claims that respiration increases with acidity in carrots.

Even the effect of phosphate concentration on respiration is not free from contradictions. LYON (9) using Elodea treated with phosphate solu-

tions at a "neutral reaction" for one hour—treatment which if prolonged to 30 hours caused death—showed that increased phosphate concentration stimulated respiration although preceded by a very brief depression. He ascribed this effect to the PO_4 ion and related the effect to the "p PO_4" (11). BOSWELL's data on the potato are different but difficult to interpret. The cation content of the buffer solutions was not specified, and during 3 hours the effect of increased phosphate concentration at pH 5.5 was to decrease respiration. Whenever a preliminary decrease in respiration of potato discs, due to phosphates of sodium or potassium, has been observed in our investigation it has always been succeeded by a prolonged period in which phosphate increased respiration. The general conclusion that phosphates decrease the respiration of potato discs should not be drawn from BOSWELL's data. LYON (11) sees in his well-known observation that inorganic phosphate will stimulate the activity of potato oxidase preparations (aqueous extracts of pulp) so that—*in presence of phosphates only*—they can oxidize sugar, evidence that the mechanism of the phosphate effect on aerobic respiration is a catalytic effect on the oxidases. Recognizing that LYON's tissue pulp extracts may have also contained nitrogen compounds, and broadening the usual range of oxidase substrates to include their secondary effects on amino-acids, such an explanation would be consistent with the standpoint of this paper.

NITROGEN METABOLISM.—Using the initial and final tissue from the experiments of tables VII and VIII, the changes which occurred in the protein nitrogen, soluble nitrogen, and the various components of the soluble nitrogen were determined by the methods which have been described. The data in absolute units are to be found in tables IX and X. The recov-

TABLE IX

EFFECT OF CONCENTRATION OF POTASSIUM PHOSPHATE BUFFER SOLUTION AT PH 6.9 ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. DURING 72 HOURS*

SAMPLE	CONCENTRATION POTASSIUM PHOSPHATE IN EXTERNAL SOLUTION	TOTAL N PER GRAM	PROTEIN N PER GRAM	PROTEIN N	SOLUBLE N	AMINO N	PERCENTAGE AMIDE N		AMMONIA† N
							STABLE	UNSTABLE	
	M	*mg.*	*mg.*	%	%	%	%	%	
Initial tissue	.....	2.02	0.59	29.2	70.8	58.4	9.4	6.4	.....
Final	0.0	2.02	0.90	44.6	55.4	44.6	5.4	7.4	.....
"	0.002	2.09	0.93	44.6	55.4	46.7	6.2	1.0	.....
"	0.010	2.05	0.99	48.3	51.7	42.9	6.8	0.5	.....
"	0.025	2.03	1.20	59.2	40.8	34.5	6.4	0.5	.....
"	0.040	2.03	1.08	53.2	46.8	38.4	7.4	0.2	.....

* Absolute units = mg. N per gm. initial fresh weight. Results on percentage basis are relative to total nitrogen.

† Negligible, order of 0.001 mg. per gm. fresh wt. No effect of conc.

TABLE X

EFFECT OF pH IN POTASSIUM PHOSPHATE BUFFERED SOLUTIONS (0.020 M PHOSPHATE) ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. DURING 72 HOURS*

SAMPLE	pH	TOTAL N PER GRAM	PROTEIN N PER GRAM	PROTEIN N	SOLUBLE N	AMINO N	AMIDE N		AMMONIA† N
							STABLE	UNSTABLE	
		mg.	*mg.*	%	%	%	%	%	
Initial tissue	.....	2.02	0.59	29.2	70.8	57.4	8.9	6.44	.....
Final	5.96	2.02	0.68	33.6	66.4	53.5	8.4	5.45	.....
"	6.48	2.00	0.83	41.5	58.5	50.0	6.5	0.55	.....
"	6.95	2.03	1.14	56.2	43.8	36.9	6.4	0.59	.....
"	7.46	2.00	0.78	39.0	61.0	54.0	8.0	0.50	.....
"	8.04	2.01	0.60	29.9	70.1	51.8	8.0	4.98	.....

* Absolute units = mg. N per gm. initial fresh weight. Results on a percentage basis are relative to total nitrogen.

† Negligible, order of 0.001 mg. per gm. fresh wt. No effect of pH.

ery of the initial total nitrogen of the tissue was quantitative and the changes which occurred are shown relative to the total nitrogen in figure 8. In this figure, protein nitrogen is represented by histograms below the line and the absolute nitrogen fractions by histograms above the line. In analyzing tissue from phosphate cultures it is essential to use the trichloracetic acid method for the determination of protein nitrogen. The alcohol method gives anomalous results since some protein is soluble in hot alcohol in the phosphate treated cultures.

The outstanding fact is that the salt or pH treatment caused respiration wherever it induced greater protein synthesis. This is shown clearly by a comparison of the histograms which represent the respiration rate (relative to standard treatments to which the value 100 is assigned) and those which represent the protein nitrogen content of the tissue (fig. 8). This is yet another, though very striking, example of the parallelism which exists between protein synthesis and respiration in potato discs. It has more than usual interest since it suggests that *even in its response to phosphate, the respiration of potato discs is modified through that same respiratory component which is linked with nitrogen metabolism.* This component is not controlled by sugar concentration, and is responsible for those other effects of inorganic salts on the respiration of potato discs that have been described in earlier papers.

The full implications of an investigation by RICHARDS (**14**) cannot be discussed here. It should be noted even though it treats of a problem apparently somewhat remote from the present one, since it correlates phosphorus deficiency during the growth of barley seedlings with low protein content and low respiration.

An explanation of the phosphate response without recourse to the hexose

phosphates and their reactions may occasion some surprise. At this stage finality is clearly impossible but the implication is that even potassium phosphates act upon metabolic processes which, as yet, appear to be somewhat remote from the metabolism of glucose via hexose phosphates. It is true that out of the increasing knowledge of the specific enzymes which

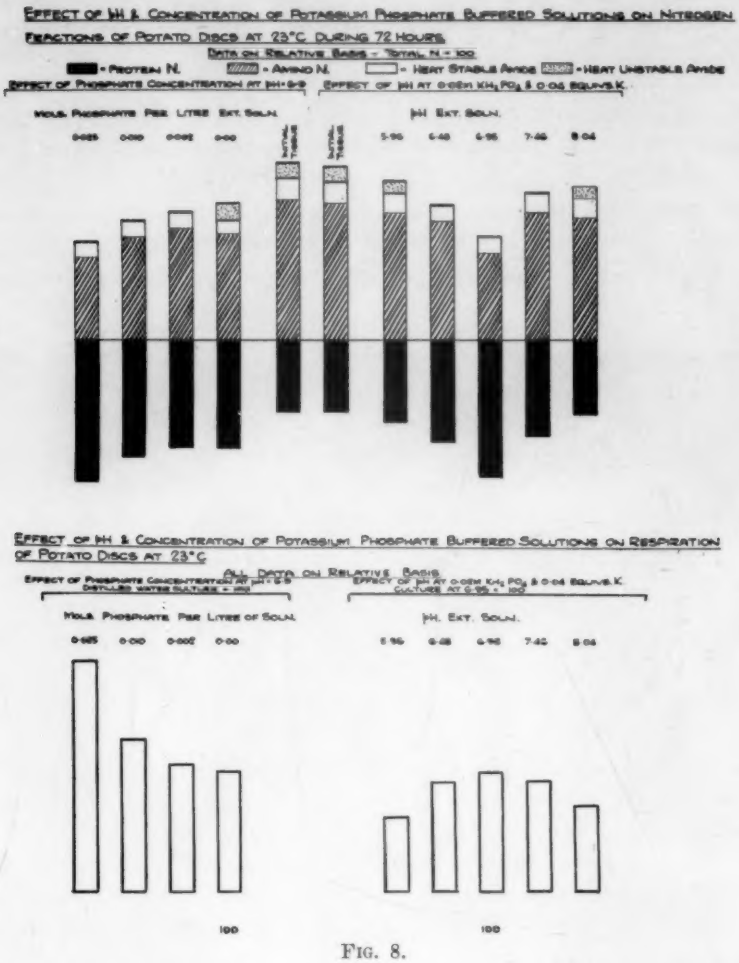
Effect of pH & Concentration of Potassium Phosphate Buffered Solutions on Nitrogen Fractions of Potato Discs at 23°C. during 72 Hours.
Data on Relative Basis - Total N. = 100
Protein N.
Amino N.
Heat Stable Amide
Heat Unstable Amide
Effect of Phosphate Concentration at pH 6·0
Effect of pH at 0·05 M KH_2PO_4 & 0·05 Equivs. K
Mols. Phosphate per Litre Ext. Soln.
pH Ext. Soln.
Effect of pH & Concentration of Potassium Phosphate Buffered Solutions on Respiration of Potato Discs at 23°C
All Data on Relative Basis
Effect of Phosphate Concentration at pH 6·0
Distilled Water Culture = 100
Effect of pH at 0·05 M KH_2PO_4 & 0·04 Equivs. K
Culture at 6·95 = 100
Mols. Phosphate per Litre of Soln.
pH. Ext. Soln.
100
100

FIG. 8.

catalyze the reactions of the hexose phosphates an explanation of the effect of pH on respiration may be forthcoming. A notable achievement is that of HANES (**4, 5**) who has isolated a phosphorylase from crude potato sap which will reversibly convert starch to glucose-l-phosphate. This enzyme has a pH optimum at 6.4, but the enzyme machinery (phosphoglucose conversion system of HANES) which converts this substance to hexosediphos-

phate and thence catalyses the splitting of the carbon chain, has apparently a pH optimum at a reaction more alkaline than pH 7.0. It is, therefore, not inconceivable that processes dependent upon the consecutive action of such enzymes might appear to be favored by a reaction of 7.0. The possible connection of the phosphorylation of sugar with deamination of amino acids and protein synthesis is remote. It still seems, however, that phosphates and pH must exert some direct effect upon nitrogen metabolism in potato discs apart from the effects exerted upon the main line of carbohydrate breakdown by the route which the latter is commonly believed to take.

In the presence of phosphate, as of other salts, the bulk of the nitrogen used in protein synthesis was drawn from the amino-nitrogen fraction; it will be seen from figure 8 that the changes in amino nitrogen are closely parallel to the increase in protein nitrogen. An outstanding feature is, however, the effect of phosphate upon that part of the total soluble nitrogen which has been designated "heat unstable amide." In the initial tissue this fraction usually comprises about one-third of the soluble nitrogen other than the true amino nitrogen. The stable amide usually decreases, but the unstable amide fraction usually increases when the tissue metabolizes in aerated distilled water (table IX, fig. 8). In all phosphate solutions with reactions between pH 6.5 and 7.5 the content of "unstable amide" in the tissue was reduced to a low level.

Comparing the tissue which was subjected to distilled water and to 0.025 M phosphate (table IX, fig. 8), it is clear that of the nitrogen for the *extra* synthesis of protein which was stimulated by the salt, approximately 70 per cent. was derived ultimately from *amino nitrogen.* The remainder, however, is accounted for by the utilization of the unstable amide fraction; this was so stimulated by phosphate that, although normally increasing in the aerated discs, it was used more rapidly than it was produced and thus the amount present in the tissue decreased. It will be recalled that the unstable amide fraction (**21, 22**) has previously been regarded as a possible reactive intermediary between amino acid and protein and this further evidence that the reserves of this substance are depleted by yet another salt treatment which stimulates synthesis and respiration is suggestive in this connection. Conversely, at those reactions (pH 6.0 to 8.0), protein synthesis and respiration were both depressed, and the stimulating effects of phosphate were no longer evident because the inherent capacity of the tissue tended to maintain its initial store of unstable amide even in contact with phosphate.

The effects of pH on protein synthesis in phosphate and bicarbonate buffered solutions can be compared by reference to figures 6 and 9. The contrast in the specific effects due to phosphate and bicarbonate concentration at constant pH is evident; and also the fact that, *under each buffer*

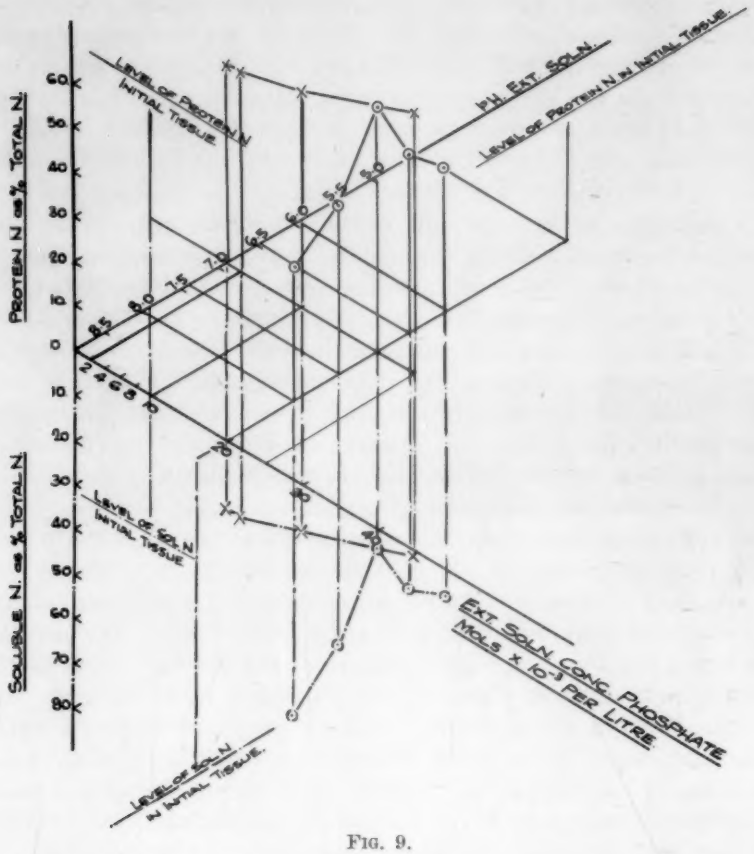
EFFECT OF pH & POTASSIUM PHOSPHATE CONC. ON PROTEIN & SOLUBLE N. OF POTATO DISCS AT 23°C & 72 HOURS
PROTEIN N as % TOTAL N.
SOLUBLE N. as % TOTAL N.
LEVEL OF PROTEIN N INITIAL TISSUE
LEVEL OF SOL N INITIAL TISSUE
pH EXT SOLN
LEVEL OF PROTEIN N IN INITIAL TISSUE
EXT SOLN CONC. PHOSPHATE MOLS x 10⁻³ PER LITRE
LEVEL OF SOL N IN INITIAL TISSUE

FIG. 9.

system, when the salt and other treatments were conducive to active protein synthesis, this reached a maximum at pH 7.0 and decreased to zero at more acid and more alkaline reactions. This effect can be attributed only to the direct effect of H^+ and OH^- upon the mechanism of protein synthesis. The obvious similarity between figure 7, which depicts the effect of pH and phosphate concentration on respiration, and figure 9 which shows the effect of similar treatments on protein synthesis, needs no further comment.

Figures 6 and 9 are reminiscent of the effect of pH upon the enzymatic synthesis of protein in concentrated protein hydrolysates. It was shown by WASTENAYS and BORSOOK (**26**) that the synthesis reached a maximum at a

pH of 4.0 in the case of egg albumin and the curve of synthesis as affected by pH was symmetrical about this reaction. They considered the possibility that this was due to the dissociation of an amphoteric component of the system although no component had an appropriate iso-electric point. The protein synthesis pH curves for potato discs are of a similar type. It is unlikely that the isoelectric properties of any amino acid present in potato determines the pH optimum at 7.0 and this appears to be attributable to some effect of the properties of water on the synthesis.

Salient features of the effect of pH, bicarbonate, dissolved carbon dioxide, and phosphates on the behavior of potato tissue

At pH 6.9, increased concentration of potassium phosphate increased both the respiration rate and protein synthesis of potato discs. In this respect phosphates behaved in a manner similar to potassium salts, in their reactions with other anions (Cl, Br, NO_3). It is thus clear that the stimulating agent is the potassium ion although its effect may be modified by the accompanying anion. Despite the prevalent idea that phosphates play an essential rôle in respiration, the stimulating effect of phosphates on the respiration of potato discs was not greater than that due to equivalent concentrations of other potassium salts. There is strong reason for the belief that the extra respiration stimulated by potassium phosphate was primarily due to that same component of respiration which is stimulated by the potassium ion as affected by other potassium salts.

At constant potassium and phosphate concentration both respiration and protein synthesis were at a maximum at an external reaction close to strict neutrality; at reactions somewhat more alkaline than pH 8.0 and more acid than pH 5.9 protein synthesis vanished. It is noteworthy that in potato discs the maximum protein synthesis does not occur in solutions at the same external reaction as that at which the extracted tissue proteins are iso-electric, *e.g.*, pH 4.4. [See Pearsall and Ewing (**13**) as suggested by Pearsall and Priestley (**12**) on theoretical grounds only.]

Potassium bicarbonate solutions in the presence of free carbonic acid and at pH 7.0 have a quite different effect upon metabolism since they depress protein synthesis and oxidase activity progressively as the salt concentration is increased. This is the *only case yet encountered in these investigations in which an increased concentration of a potassium salt decreased the respiration and metabolism of potato discs. It is evident that this effect is due specifically to* HCO_3^- *and* H_2CO_3. Although the available data are limited, it is clear that the metabolism of carbohydrate and respiration were also depressed by these salt treatments; the trend of the effect of bicarbonates and pH upon these processes should be similar to that found, and more fully investigated, for protein synthesis.

Increased concentrations of potassium bicarbonate and free acid at constant pH depress, and eventually suppress altogether, *both protein synthesis and also the accumulation of the bromide ion.* These facts again imply that the processes of *respiration, protein synthesis, and bromide uptake in potato cells are all closely linked together.* The data also show, however, that potassium was absorbed even from bicarbonate solutions which suppressed protein synthesis and bromide uptake. The presumption is that this was accompanied into the tissue by bicarbonate, which then reacted with the organic acids of the tissue since the bicarbonate concentration of the sap did not increase. Such absorption of potassium seems not to be as essentially related to growth as the simultaneous uptake of potassium and bromide. Treatment with solutions of bicarbonate and free carbonic acid affects the potassium previously stored in the potato cells in a manner which suggests that they *retain their salts most effectively under conditions which are optimum for protein synthesis.* All the evidence, therefore, reinforces the view that protein metabolism plays an essential rôle in the production and maintenance of ionic accumulation gradients.

The depressing effect of bicarbonates upon metabolism and bromide uptake cannot be ascribed wholly to the combined carbonic acid or to the free acid but both play a part. It seems that the bicarbonate ion present is more potent than the undissociated free acid. Three-dimensional models portray the inter-related effects of pH, bromide absorbed in the sap, protein synthesis, and potassium bicarbonate (total carbonic acid concentration in the solution). These show that the influence of added bicarbonate and free carbonic acid on bromide uptake or protein synthesis reaches a maximum at pH 7.0. Similarly, protein synthesis is, at any given salt ($KHCO_3$) concentration, at a maximum at pH 7.0 and declines to zero at reactions as alkaline as pH 8.7 and as acid as pH 5.3.

The almost identical effect on protein synthesis of solutions of different pH but constant salt concentration which were obtained with the different buffer systems employed, although the constituent salts (bicarbonate and phosphate) have distinct and opposed effects on metabolism, is evidence that the effect of pH at constant potassium concentration which is in question *is mainly due to the H and OH ions* and not to other ions or molecules in the buffer systems concerned. The importance of the reaction pH 7.0, at which protein synthesis is at a maximum and the depressing effect of added bicarbonate a minimum, is an indication that the process is limited in some way by the properties of water; the physico-chemical properties of plant amino-acids and proteins (iso-electric points) do not show maxima or minima at a pH of 7.0.

The effects of both phosphate and bicarbonate upon the nitrogen metabolism of the discs ultimately become apparent upon the utilization of the

amino-acid reserves. In both cases, the color reactions which the treatments induce, suggest that the activity of the phenolase in the tissue is a potent part of the mechanism of the salt effects. In both cases, however, there is evidence which suggests that the effect of the salts extends beyond the possible rôle of the phenolase in the deamination of amino compounds. The unstable, amide-like substance, to which previous reference has been made, is so affected by phosphate and bicarbonate treatments that a relative increase in protein synthesis caused by the salt results in a relative decrease of unstable amide and vice versa. On the highly probable assumption that the substance in question is formed from sugar and the products of deamination of amino acids and is an intermediate in protein synthesis, it becomes clear that *the effects of phosphate and bicarbonate extend to the later stages of the conversion of such intermediates to protein and are not confined to the deamination mechanism alone.* Of course, phenolase may also be involved in these stages.

Despite the fact that metabolic processes (respiration and protein synthesis), which are clearly connected with the ability of the tissue to absorb salts, are favored by a neutral reaction of pH 7.0 there is, nevertheless, a wide range of pH (6 to 8) within which the bromide uptake is not conspicuously affected by pH.

The investigation emphasizes again the ramifications of the biochemical effects due to ions in the external solution which extend to all phases of the metabolism of the tissue investigated and which are seen to be characteristic of all the ionic species that have been considered in this and preceding papers.

Summary

1. The effect of pH and the concentration of a $KHCO_3/H_2CO_3$ buffer on the absorption of bromide and the metabolism of potato discs has been investigated using thin discs in aerated solutions at 23° C.

2. Throughout, the effects of these treatments on the uptake of bromide, on protein synthesis, and on oxidations in the tissue catalyzed by oxidases (phenolase) are closely parallel.

3. At constant pH, increasing the external concentration of $KHCO_3$ and dissolved carbon dioxide depresses (and eventually suppresses completely) both protein synthesis and bromide accumulation. To this effect both combined and free carbonic acid contribute; at pH 7.0, their relative effects are about equal. Of HCO_3^- and undissociated H_2CO_3, the former appears to have the greater effect on the tissue.

4. There is a relatively broad pH range, in the absence of bicarbonate, within which bromide uptake is not much affected by $[H^+]$ and its attendant variables. In the presence of bicarbonate, protein synthesis (though depressed by the salt) is at its maximum at pH 7.0 and is less active in a more

acid or alkaline solution. These effects are specifically due to H^+ and OH^- and find a parallel in bromide uptake which is depressed by bicarbonate solutions and is more favored under these conditions by a pH of 7.0 than by reactions more acid or more alkaline.

5. The effect of HCO_3^- and H_2CO_3 on nitrogen metabolism is also shown by their effect on the utilization of amino acids but it extends to subsequent reactions; *e.g.*, reactions in which unstable amides are involved as intermediaries of protein synthesis.

6. The effects of pH and the concentration of a bicarbonate buffer on the behavior of the tissue (bromide uptake, nitrogen metabolism, etc.) can be represented by 3-dimensional figures. Comparison of these figures shows that bromide uptake and protein synthesis from soluble nitrogen reserves, are similarly affected by these variables.

7. The evidence shows again that in neutral solution (strictly pH 6.9) K^+ and Br^- are equally absorbed by potato discs. At more acid reactions (by CO_2), conditions which are also less favorable for protein synthesis, the tissue loses potassium. The best conditions for protein synthesis are conducive to the maintenance of existing concentrations in the cell sap.

Tissue, however, in which neither bromide uptake nor protein synthesis occurred, absorbed potassium from relatively strong bicarbonate solutions. This special case of potassium absorption (accompanied by HCO_3^- which apparently reacts with the organic acids of the cell sap) is less dependent on the processes of growth than the case in which a cation and a non-reactive anion are absorbed together.

8. Indirect evidence shows that the bicarbonate solutions which retard bromide absorption and protein synthesis also depress respiration and the metabolism of carbohydrate.

The mechanism of the effect of HCO_3^- and H_2CO_3 on metabolism is briefly discussed.

9. The effect of pH and phosphate concentration on respiration and nitrogen metabolism has been investigated using aerated phosphate buffers of constant potassium content.

At pH 7.0, increased concentration of a phosphate buffer increases both respiration and protein synthesis in potato discs.

At constant phosphate concentration *both* respiration and protein synthesis are at a maximum at pH 7.0 and decrease in solutions more acid and more alkaline.

The similarity in the effects of these variables on both respiration and nitrogen metabolism is seen by comparing figures which show simultaneously the variation of the property in question (respiration, protein synthesis, or loss of soluble nitrogen) in relation to two variables (pH and phosphate concentration).

10. Phosphate treatments which stimulate respiration deplete the tissue of the unstable amide, which is normally present in discs of potato in aerated solution and which appears to be a reactive intermediary in protein synthesis.

11. Despite the different specific effects attributable to the phosphate and bicarbonate the parallelism between protein synthesis and respiration is consistent throughout. Although phosphates increase and bicarbonates decrease respiration the effect of pH is similar in both buffer systems. These effects must be ascribed specifically to H^+ or OH^- and, if not due to specific enzymes with definite pH optima, their symmetry about pH 7.0 suggests that they are determined by properties of water and not of the ampholytes (amino acids, proteins) concerned.

Brief reference is made to the general problem of the effect of pH on respiration and to discrepancies in the literature.

12. Phosphates seem to influence metabolism of potato discs through their effect on nitrogen metabolism (protein synthesis and use of amino acids) ; *i.e.*, processes which appear remote from the accepted rôle of hexose phosphates in carbohydrate breakdown.

13. Respiration, protein synthesis, and bromide absorption are again seen to be closely linked. They are similarly affected by external variables, the effect of which on the tissue is consistently shown by reactions stimulated by the oxidase (phenolase) it contains.

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LITERATURE CITED

1. Boswell, J. G., and Whiting, G. C. A study of the polyphenol oxidase system in potato tubers. Ann. Bot. n.s. **2**: 847–868. 1938.
2. Clark, W. M. Determination of hydrogen ions. Baltimore. 1928.
3. Fife, J. M., and Frampton, V. L. The effect of carbon dioxide upon the pH and certain nitrogen fractions of the sugar beet plant. Jour. Biol. Chem. **109**: 643–655. 1935.

4. HANES, C. S. The breakdown and synthesis of starch by an enzyme system from pea seeds. Proc. Roy. Soc. London B **128**: 421–450. 1940.
5. ————————. Enzymic synthesis of starch from glucose-I-phosphate. Nature **145**: 348–349. 1940.
6. HOAGLAND, D. R., and BROYER, T. C. Hydrogen-ion effects and the accumulation of salt by barley roots as influenced by metabolism. Amer. Jour. Bot. **27**: 173–185. 1940.
7. HOLT, L. E., LAMER, V. K., and CHOWN, H. B. The solubility product of secondary and tertiary calcium phosphate under various conditions. Jour. Biol. Chem. **64**: 509–565. 1925.
8. LEMMON, P. Respiration of potato tissue in relation to hydrogen ion concentration of a surrounding solution. Amer. Jour. Bot. **23**: 296–302. 1936.
9. LYON, C. J. Effect of phosphates on respiration. Jour. Gen. Physiol. **6**: 299–306. 1924.
10. ————————. Phosphate ion as a promoter catalyst of respiration. Jour. Gen. Physiol. **10**: 599–615. 1927.
11. ————————. The rôle of phosphate in plant respiration. Amer. Jour. Bot. **14**: 274–283. 1927.
12. PEARSALL, W. H., and PRIESTLEY, J. H. Meristematic tissues and protein iso-electric points. New Phytol. **22**: 185–191. 1923.
13. ————————, and EWING, J. The iso-electric points of some plant proteins. Biochem. Jour. **18**: 329–339. 1924.
14. RICHARDS, F. J. Physiological studies in plant nutrition. VIII. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaf as determined by phosphorus and potassium supply. Ann. Bot. n.s. **2**: 491–534. 1938.
15. STEWARD, F. C. A technique for the study of respiration and salt absorption in storage tissue under controlled environmental conditions. Protoplasma **15**: 497–516. 1932.
16. ————————. Observations upon the effects of time, oxygen and salt concentration upon absorption and respiration by storage tissue. Protoplasma **18**: 208–242. 1933.
17. ————————. Mineral nutrition of plants. Ann. Rev. Biochem. **4**: 519–544. 1935.
18. ————————. Salt accumulation by plants—the rôle of growth and metabolism. Trans. Faraday Soc. **33**: 1006–1016. 1937.
19. ————————, BERRY, W. E., and BROYER, T. C. The effect of oxygen upon respiration and salt accumulation. Ann. Bot. **50**: 346–366. 1936.
20. ————————, and HARRISON, J. A. The absorption of rubidium bromide by potato discs. Ann. Bot. n.s. **3**: 427–454. 1939.

21. ————————, and PRESTON, C. Metabolic processes of potato discs under conditions conducive to salt accumulation. Plant Physiol. **15**: 23–61. 1940.

22. ————————, and ————————. The effect of salt concentration upon the metabolism of potato discs and the contrasted effect of potassium and calcium salts which have a common ion. Plant Physiol. **16**: 85–116. 1941.

23. ————————, STOUT, P. R., and PRESTON, C. The balance sheet of metabolites for potato discs showing the effect of salts and dissolved oxygen on metabolism at 23° C. Plant Physiol. **15**: 409–447. 1940.

24. THOMAS, L. A. The respiration of carrot tissue in buffer solutions. Australian Jour. Exp. Biol. Med. Sci. **8**: 225–236. 1931.

25. THORNTON, N. C. The influence of carbon dioxide on the acidity of plant tissue. Contrib. Boyce Thompson Inst. **5**: 403–418. 1933.

26. WASTENEYS, H., and BORSOOK, H. Enzymatic synthesis of protein. III. The effect of hydrogen ion concentration on peptic synthesis. Jour. Biol. Chem. **62**: 675–686. 1938.

27. WILLIAMS, J. J., and BEAUMONT, J. H. The effect of accumulated carbon dioxide on plant respiration. Plant Physiol. **3**: 45–59. 1928.

ACCUMULATION OF ARSENIC IN THE SHOOTS OF SUDAN GRASS AND BUSH BEAN[1]

LEONARD MACHLIS

(WITH SIX FIGURES)

Arsenic, like certain other elements, is toxic to plant and animal life in relatively minute quantities. It has no effect on, or may possibly be beneficial to, plants when present in traces (**3, 12, 24, 25**). Since it is an active poison the extensive use of arsenical herbicides and insecticides has been attended by several hazards.

A small part of the toxicants adheres to the sprayed or dusted fruits and vegetables, thus rendering them inedible unless washed. Legislation now limits the amount of spray residue allowed to remain on these plant products. The greater portion of the applied arsenicals are eventually deposited in the soil where they accumulate in the surface layer (**14, 17, 27**) in quantities sufficient to retard plant growth. Unsuccessful crop production because of accumulated arsenical residues has occurred in South Carolina (**1, 7**), Louisiana (**22**), and Washington (**27**).

In 1937 JONES and HATCH (**17**) suggested that plants growing on arsenated soils might absorb quantities of arsenic which would be deleterious to livestock and humans if such plants were ingested. They found only the normal trace content of arsenic in apples grown on soils high in arsenic as a result of regular spraying practices. The negligible arsenic content of the apples was attributed to the fact that most of the roots were located below the surface layer of the soil in which the accumulated arsenic residues were found.

The investigation reported below was designed primarily to determine the extent of accumulation of arsenic in agricultural plants having access to the element and to assess the data with respect to growers and consumers of plant products.

Data on the arsenic content of plants grown under various conditions are meager. Analyses of plants growing on soils containing naturally-occurring arsenic have shown traces to be present (**16, 24, 26**). These are, however, insignificant as well as unavoidable.

Two reports in the literature indicate that high-arsenic soils can produce high-arsenic plants. FELLENBERG (**11**) describes a meadow in the vicinity of Buus, Germany, which he found to contain 700 to 800 times as much arsenic as did cultivated fields in the surrounding country. The plants growing on this soil were also high in arsenic. He writes: "Horses, accord-

[1] Published by permission of the Director of the Hawaii Agricultural Experiment Station as technical paper no. 72.

ing to statements by tenants of Erzmatt, do not eat the hay, and sheep must not be fed with it except mixed with other hay, for otherwise they will become sick. Sheep even have become slightly sick when grazing. Consequently, we are dealing here with amounts of arsenic which already have a toxic effect upon these animals.''[2] It should be noted that this soil had a very scanty flora and that attempts to grow different cultivated plants ended in failure. VANDECAVEYE, HORNER, and KEATON (**27**) grew barley plants on soils obtained from the surface six inches of representative areas in each of three fields in the Yakima Valley, Washington. Two attempts to grow crops of barley and alfalfa on two of these soils failed. These had previously been in orchards which had been sprayed with lead arsenate over a period of years. The third had never been in orchard and was productive. The tops from barley plants grown on the unproductive soils yielded 10.01 and 17.50 p.p.m. As_2O_3 respectively, on the dry weight basis; only a trace was found in the plants grown on the productive soil. The plants high in arsenic grew very poorly.

These examples indicate that some plants are capable of absorbing considerable quantities of arsenic from soils which have high arsenic contents. Such soils occur in the cotton belt (**1, 21, 22**), orchard growing regions (**14, 27**), and probably wherever arsenicals have been used over a period of years. They are a potential source of arsenic in plant food products.

From the point of view of this paper, a high arsenic content alone is of no significance. When different plants are grown in increasing concentrations of arsenic of the order of 0 to 25 p.p.m. there is a progressive decrease in growth (measured by weight) up to a lethal concentration which varies with the plant species (**3**). Exceedingly low concentrations of arsenic do not retard growth. For each plant, then, there is a range of concentrations of arsenic in which growth and accumulation of the element may take place. It is clear that good growth and extensive accumulation must take place concurrently if this phase of the general arsenic problem merits serious consideration. Studies were begun in 1937 at this station to obtain information pertinent to the problem. This report presents certain experiments in which water cultures were employed.

Materials and methods

The two plants selected for study were Sudan grass (*Sorghum vulgare* var. *sudanense*) and bush bean (*Phaseolus vulgaris* var. *humilis*). The former is used as hay and ensilage and the latter is raised for its fruit. Each plant was grown in two different but overlapping series of concentrations of sodium arsenite ($NaAsO_2$) which are listed in table I. One series

[2] This quotation and a later one are from a translation for which the writer is indebted to MR. F. THOMPSON, translator for the Division of Plant Nutrition, University of California, Berkeley.

TABLE I

EFFECT OF THE CONCENTRATION OF ARSENIC IN THE CULTURE MEDIUM ON THE DRY WEIGHT YIELD OF SUDAN GRASS AND BEAN*

SUDAN GRASS SERIES I. GROWTH PERIOD: 63 DAYS					SUDAN GRASS SERIES II. GROWTH PERIOD: 58 DAYS				
CULTURE CONCENTRATION OF As	YIELD OF TOPS	YIELD OF TOPS‡	YIELD OF ROOTS	YIELD OF ROOTS‡	CULTURE CONCENTRATION OF As	YIELD OF TOPS	YIELD OF TOPS‡	YIELD OF ROOTS	YIELD OF ROOTS‡
p.p.m.	*gm.*	%	*gm.*	%	*p.p.m.*	*gm.*	%	*gm.*	%
0.0	89.0	100	11.9	100	0.00	50.0	100	9.4	100
1.0	66.1	74	10.6	89	0.25	55.2	110	10.3	110
2.0	49.1	55	10.2	86	0.50	50.1	100	10.1	107
3.0	42.9	48	8.1	68	0.75	47.4	95	9.3	99
4.0	30.9	35	7.1	59	1.00	44.2	88	9.8	105
5.0	35.7	40	8.1	68	1.25	38.5	77	9.5	102
6.0	30.8	35	7.4	62	1.50	43.9	88	9.3	99
7.0	20.3	22	5.4	45	1.75	41.8	83	9.3	99
8.0	25.2	28	6.0	51	2.00	42.3	85	8.8	94
9.0	18.7	21	4.4	37	2.50	35.3	75	8.2	88
10.0	20.2	22	5.0	42	.....	.....	.....	.....	.....
12.0	11.7	13	3.7	31	.....	.....	.....	.....	.....
14.0	5.0	5	1.5	12	.....	.....	.....	.....	.....
16.0	4.9	5	1.3	10	.....	.....	.....	.....	.....
18.0	0.0	0	0.0	0	.....	.....	.....	.....	.....
BEAN SERIES I.† GROWTH PERIOD: 4 DAYS					BEAN SERIES II. GROWTH PERIOD: 29 DAYS				
0.00	11.0	100	.....	.....	0.0	14.2	100	7.0	100
0.25	6.4	58	.....	.....	0.1	16.0	112	8.1	116
0.50	4.6	42	.....	.....	0.2	13.0	92	7.0	99
0.75	2.8	26	.....	.....	0.3	16.3	114	9.1	129
1.00	2.2	20	.....	.....	0.4	19.0	131	10.7	153
2.00	0.4	4	.....	.....	0.5	13.4	95	8.1	116
3.00	−0.7	−6	.....	.....	0.6	14.2	100	8.2	119
4.00	−0.1	−1	.....	.....	0.7	9.6	68	6.8	96
5.00	0.0	0	.....	.....	0.8	7.3	52	5.0	71
6.00	−0.8	−7	.....	.....	1.0	6.4	45	4.1	55
7.00	−0.3	−3	.....	.....	1.2	2.2	15	2.7	38
8.00	−0.2	−2	.....	.....	1.6	0.5	4	1.5	21
9.00	0.7	6	.....	.....	1.0	0.4	3	1.6	22
10.00	0.7	6	.....	.....	3.0	0.0	0	1.1	16

* The yields recorded are for a single plant for Sudan grass; 10 plants for bean series I; single plant for the tops of bean series II; and 5 plants for the roots of bean series II.

† The negative values for bean series I are caused by variation in the weights of the seedlings at the beginning of the arsenic treatment.

‡ Percentage of control.

covered the entire range of concentrations in which each plant could live; the second contained a larger number of concentrations in the non-toxic or slightly toxic range. The terms "toxic" and "toxicity" are used in the sense of any repression of growth as measured by the dry weights of the

plants. The arsenic content of the stem, leaf, node, etc., of the Sudan grass plants at several intervals between the seedling and flowering stages and of the bean plants for a single collection was determined.

Each series was grown in duplicate. With the exception of bean series I all data are averages of two independently determined values which did not vary sufficiently to affect the present interpretation. The arsenic content of the root was not systematically determined owing to the difficulty of distinguishing between arsenic held internally and externally.

Culture methods

The plants were grown in 17- and 20-liter crocks on stationary tables in a greenhouse on the University of Hawaii campus. Each crock was fitted with a varnished plywood cover cut to hold six or eight flat corks which, in turn, were bored to hold a maximum of five plants each. Sudan grass seeds were germinated on cheesecloth supported over water in enameled pans and the bean seeds between sheets of moist paper. Selected seedlings, held in the corks with nonabsorbent cotton, were grown in arsenic-free nutrient solution for 7 to 10 days. At the end of this period the nutrients were renewed and the required amounts of arsenic added to each culture from a stock solution of $NaAsO_2$. Both nutrients and arsenic were thereafter renewed weekly for the duration of each experiment. Occasional analyses of the solutions showed that the arsenic concentration decreased slightly during the week from the calculated initial concentrations. Thus, when the latter were 0.25, 1.0, 5.0, 10.0, and 18.0 p.p.m. As, the concentrations at the end of the week were found to be 0.14, 0.72, 4.62, 9.56, and 17.22 p.p.m. As, respectively. When the plants had attained considerable size, suitable support was provided to keep them upright. The environmental conditions were equalized as much as possible by varying the positions of the cultures.

Hoagland's nutrient solution consisting of 0.005 M KNO_3, 0.005 M $Ca(NO_3)_2$, 0.002 M $MgSO_4$, and 0.001 M KH_2PO_4 was used for Sudan grass and a three-salt solution composed of 0.005 M $Ca(NO_3)_2$, 0.002 M $MgSO_4$, and 0.002 M KH_2PO_4 for the bean plants. To both solutions $FeSO_4$, $MnSO_4$, and H_3BO_3 were added in small quantities. The experiments with bean were done following those with Sudan grass. The change to the three-salt solution was made because this nutrient was easier to prepare and produced healthy plants. It is considered unlikely that the changes in the concentrations of the salts were of magnitudes that could affect the interpretation of the data.

These solutions were made up in quantities of 100 liters and transferred to the crocks in a galvanized iron container designed to deliver either 17 or 20 liters of solution. Baker's C.P. grade chemicals, distilled water, and

continuous forced aeration were used in all of the experiments. Normal H_2SO_4 was added as needed to maintain the pH between 4 and 5. The plants were sprayed occasionally with nicotine sulphate (1:800) to control thrips and aphids.

Plan of experiments

Sudan grass series I.—Thirty seedlings, nine days old, were placed in each crock (17-liter capacity) containing arsenic-free nutrient solution on Oct. 12, 1938. One week later the solutions were renewed and the required amounts of arsenic added. On Oct. 26, Nov. 2, 9, and 23, and Dec. 7, and 21, respectively, one cork of five plants was removed from each culture, weighed, and prepared for analysis. The plants were divided into the following parts: Oct. 26 and Nov. 2 collections into root and plant top; subsequent collections into root, tillers, and the primary shoot which was further divided into node, internode, leaf, and inflorescence, if present.

For the purpose of this study the plant top of Sudan grass was divided into the main axis, or primary shoot, and the tillers or secondary shoots that arise from axillary buds at the basal nodes of the main axis. The latter produce their own adventitious root systems. The division was made for two reasons: (1) the tillers are of variable size and consequently difficult to dissect completely into their several morphological parts; and (2) they are produced at all times during the experimental period, whereas the primary shoot is present from the beginning. In the text the tillers plus the primary shoot are referred to as the plant top. The nodes, leaves, internodes, and inflorescences are those of the primary shoot only.

Sudan grass series II.—Forty seedlings, 7 days old, were placed in each crock (20-liter capacity) on Oct. 21. One week later the solutions were renewed and the arsenic added. One cork of five plants was removed from each culture on Nov. 4, 11, 18, and 25, and on Dec. 2, 9, 16, and 24, weighed, and prepared for analysis. The plants were divided as follows: Nov. 4 and 11 collections, into root and plant top; Nov. 18 and 25 into root, tillers, and primary shoot; and the remaining collections into root, tillers, node, internode, leaf, and inflorescence as above. The Dec. 2 and 16 collections were not analyzed for arsenic.

Bean series I.—The plants in this series did not survive in concentrations of arsenic in excess of 2 p.p.m.; consequently, it was discontinued 4 days after the addition of arsenic to the cultures. Five seedlings, 3 days old, were introduced into each crock (17-liter capacity) on Jan. 15, 1939. The solutions were changed and the arsenic added on Jan. 26 and four days later the plants were harvested. The plants from duplicate cultures were composited, and only the shoots prepared for analysis without further division.

Bean series II.—Five seedlings, five days old, were placed in each crock

(17-liter capacity) on Feb. 4. The required amounts of arsenic were added Feb. 16. One month later the plants were harvested, divided into root, stem, petiole, leaf blade, and fruit, and prepared for analysis.

Analytical methods

The fresh plant material was cut into small pieces and dried to constant weight in a blast of hot air at 85° to 95° C. The larger samples were ground in a Wiley mill, the smaller in a drug mill; both were stored in paper envelopes. Prior to analysis, the opened envelopes were placed in an electric oven at approximately 60° C. for six hours or more and then cooled in a desiccator.

The official digestion method used in the Gutzeit procedure (**20**), modified by the substitution of perchloric acid for ammonium oxalate in the driving off of nitrogen oxides (**5**), was adapted to the small quantities of material available. The procedure used was as follows: Two grams or less of the dried ground sample were weighed into 125-ml. Erlenmeyer flasks which had previously been calibrated and marked at the 125-ml. level. To the aliquot, 1 to 2 ml. of H_2O, 20 ml. of HNO_3, and 10 ml. of H_2SO_4 were added in order. These were shaken carefully two or three times over a 15-minute period. When the reaction subsided, the flasks were covered and allowed to stand for several hours to prevent excessive foaming when subsequently heated on an electric hotplate. During the heating 5 to 10 ml. portions of HNO_3 were added to the solutions until, with the evolution of SO_3 fumes, a clear white or amber color obtained. The flasks were then removed from the hotplate, allowed to cool slightly, and 2 ml. of 70 per cent. perchloric acid were added. After this, heating was continued until the evolution of dense SO_3 fumes. They were cooled again and made up to 125 ml. Sixty samples can be digested in this way in about three hours with a sufficiently large hotplate.

In the estimation of arsenic, the titration method of Cassil and Wichmann (**6**) was used with the exception that the maximum quantity of arsenic determined was limited to 50 micrograms As_2O_3. Arsenic is reported as As unless otherwise designated.

The inherent standard deviation of a single analysis was found from quadruplicate analyses of four samples of arsenic-containing plant material. The amounts found (as micrograms As_2O_3 per gram of dry material) and the standard deviations (corrected for small samples) were 17.68 ± 0.26, 26.67 ± 0.70, 32.25 ± 0.58, and 51.68 ± 0.46. The average of the standard deviations over the entire range was ± 1.79 per cent.

Time did not permit a critical determination of the percentage recovery of arsenic over the range used (**6**). Estimates based on limited tests indicate that in aliquots containing from 5 to 50 micrograms As_2O_3 the amount found may be 5 per cent. less than the actual amount and in aliquots con-

taining less than 5 micrograms the amount found may deviate as much as ± 10 per cent. from the actual amount. Affected by the latter error are 15 per cent. of the values presented for the arsenic contents of the plant parts of Sudan grass series I and II and 80 per cent. of the values for the plant parts of bean series II.

Results

Growth of the plants

Yield.—The yield is here considered to be the dry weight of plant material produced in each culture subsequent to the initiation of the arsenic treatment. The data for each series are presented in table I. It should be noted that the yields of Sudan grass, as defined above, represent a summation of the weights of the plants removed for the several collections from any one culture. This method was adopted to equalize variation between the weights of the plants in a culture.

With reference to the plant tops, concentrations of arsenic below 0.5 to 0.6 p.p.m. were non-toxic to both plants and may have been beneficial, while above this the yield was progressively reduced as the arsenic approached the lethal concentrations of 18 and about 2 p.p.m. of As, for Sudan grass and bean, respectively. The roots were similarly affected but in all concentrations they produced relatively more dry material than the tops.

The maximum concentrations of arsenic in which these plants can grow are probably closer to 1.2 and 10.0 p.p.m. for bean and Sudan grass, respectively. The roots of plants growing in concentrations in excess of these were almost immediately killed and were thus non-functional. Under this condition, the water, nutrients, and arsenic would enter directly into the transpiration stream, which probably accounts for the slight growth of the tops observed. Under soil conditions it is unlikely that such plants could have survived. Out of a total of 60 Sudan grass plants in the duplicate cultures containing 12, 14, 16, and 18 p.p.m. As only 53 per cent., 23 per cent., 10 per cent., and 0 per cent., respectively, survived the first week of treatment.

The concentrations, 1.2 and 10 p.p.m. As for bean and Sudan grass, respectively, determine the maximum concentrations of arsenic from which accumulation may take place by each plant. These relative sensitivities are directly applicable to soil conditions and apparently are representative of closely related plant species. Thus Brenchley (**3**) found that in water cultures 4 p.p.m. arsenious acid prevented the growth of peas and 20 p.p.m. that of barley. Morris and Swingle (**18**) observed that when several different arsenicals were added to soil plots, beans were killed; barley grew uninjured. Their report also indicated that some differences in sensitivity are to be expected between related plant species. Stewart and Smith (**24**)

observed that beans seemed decidedly more sensitive than wheat and other plants growing in soil to which disodium arsenate had been added. Peas were more resistant than beans and potatoes, and seemed more sensitive than wheat.

The high yields of some of the plants growing in the very weak concentrations of arsenic, although probably not statistically significant, suggest a beneficial function for arsenic. This may be the indirect effect of the action of the arsenic on the fauna and flora of the cultures or it may indicate the essentiality of the element. Increased growth or healthier appearing plants have been reported where arsenic was added to soil plots (**12, 24, 25**); BRENCHLEY (**3**), however, using water cultures, was unable to find any evidence indicating a beneficial action of arsenic.

If the criteria of ARNON and STOUT (**2**) for essentiality are accepted, none of the experiments, including those reported herein, have demonstrated to date a requirement of plants for arsenic.

OTHER RESPONSES.—The first recognizable response of the plants to the arsenic treatment was a wilting of the leaves of both species. This occurred daily for a period of several days with a recovery of turgescence at night. The extent of wilting of the plants growing in the various concentrations of arsenic was more or less proportional to the toxicity of the arsenic to the plants as indicated in table I and recovery was inversely so. The wilting took place very quickly. For example, arsenic was added to the cultures of bean series II at 1:30 P.M. on a sunny day and by 3:30 P.M. some of the leaflets of the plants growing in the highest concentrations of arsenic were wilting. About 10:00 A.M. the next morning all of the plants in solutions containing 0.5 p.p.m. or more As displayed some degree of wilt. The Sudan grass plants responded in a similar manner.

Two to three days after the introduction of arsenic into the solutions necrotic leaf tissue was observed. The killed portions were the tips of the Sudan grass leaves and the margins of the bean leaves. The extent of killed tissue increased with increasing concentrations of arsenic in the cultures. The daily wilting decreased in severity as the plants became older. After two or three weeks a noticeable wilt was observed only on the day or two immediately following the renewal of the arsenic and nutrients.

The leaf injury took place predominantly during the first week of growth in the arsenic-containing solutions. New leaves produced in all of the Sudan grass cultures and in most of the bean cultures subsequent to the initial injury were for the most part green and healthy. This was noteworthy because, as will be shown later, these apparently healthy leaves contained high concentrations of arsenic.

It was observed that the roots became flaccid—again in proportion to the toxicity of the arsenic to the plant. Those in the very high concentrations

(above 1.2 and 12 p.p.m. As for bean and Sudan grass, respectively) soon became gelatinous and disorganized.

The root systems tended to become red two or three days after the additions of arsenic to the cultures. Those of the bean and Sudan grass plants growing in concentrations of less than 3 p.p.m. of As were tinged a slight pink. The color did not become noticeably more intense with time. The Sudan grass roots in the higher concentrations soon became a dark red, particularly the larger roots, with the exception of the roots tips. The root systems of the bean and Sudan grass plants that were observed to become gelatinous did not become discolored, suggesting that they were killed before the reactions resulting in the reddening could take place. The cortical tissues were most severely affected for they could be removed leaving the white stele with its attached, small, lateral roots.

In brief, it seems that the arsenic effected a sudden decrease in the movement of water into the plants. As a result of this interference with the supply of water to the plant top the leaves wilted and died back. MORRIS and SWINGLE (**18**) working with both water cultures and soils, observed that the addition of arsenicals to the growth media was followed by a marked decrease in transpiration. FELLENBURG's description (**11**) of plants growing on a high-arsenic soil also suggests that arsenic interferes with the water relations of plants.

The reduced yields of bean and Sudan grass may be a response to this physiological drought; however, arsenic was found throughout the plants and could inhibit growth by interference with other processes.

It is significant that no injury or abnormality of the plant tops was observed that was characteristic for arsenic poisoning. The wilting and subsequent killing of part of the leaves, as well as the decreased yield of the plants, apparently differed in no way from similar responses to be expected from a sudden drought.

Analytical data

CONCENTRATION OF ARSENIC IN THE PLANT TOPS

In figure 1 the weighted average concentrations of arsenic found in the plant tops of Sudan grass series I at the time of each collection are presented graphically. It is to be noted that over the range of 0 to 8 p.p.m. As in the culture solutions the concentration of arsenic in the plant top is a straight-line function of that in the nutrient solution and, secondly, that it remains constant with time for each culture. The widely fluctuating values for the plants growing in the high concentrations of arsenic (9 to 18 p.p.m. of which those above 12 are not plotted because the range of values was too extensive to get into the figure) are indicative of the previous observation that the roots of these plants were non-functional.

Similar data for Sudan grass series II are substantially a reproduction of those of series I over the corresponding range of solution concentrations. There was a tendency for the concentrations of arsenic within the plant tops of series II to decrease during the course of the experiment; the magnitudes of these changes were necessarily small, however. Data are presented for the Dec. 9 collection (table V) representing a series of concentrations midway between the highest and lowest observed during the experimental period. These data for series II are to be compared with the Dec. 21 collection of series I (table IV) which is representative of the concentrations found in the plant tops of series I throughout the experimental period.

In a like manner, the concentrations of arsenic in the plant tops of the two bean series are substantially the same for those plants growing in equal concentrations of arsenic even though bean series I was conducted for 4 days and bean series II for 30 days (tables VI, VII).

The values found in each of the four series are compared in table II.

TABLE II

CONCENTRATION OF ARSENIC IN THE PLANT TOPS OF BEAN AND SUDAN GRASS AS PARTS PER MILLION OF ARSENIC, DRY WEIGHT BASIS

Culture concentration	Bean series I	Bean series II	Sudan grass series I	Sudan grass series II	
	4 days	30 days	63 days	42 days	56 days
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
0.25	3.6	.....	.....	3.9	1.3
0.50	5.2	3.9	.....	4.7	2.9
0.75	3.9	.....	.....	7.4	4.3
1.00	4.3	8.4	9.6	7.8	5.1
2.00	17.9	20.0	14.7	15.6	11.2

The comparison is obviously restricted to those plants growing in equal concentrations of arsenic. Values from two different collections of Sudan grass series II are presented owing to the tendency for the arsenic content of these plants to decrease with time. It is seen that irrespective of the plant species or of the length of the growth period in the arsenic-containing medium the arsenic concentrations in all of the plants are of the same order of magnitude for each concentration of arsenic in the nutrient solutions.

The data indicate that arsenic readily enters the plants. The concentration in the plant top is governed, apparently, by that in the nutrient solution; hence, the sensitivity of the plant to arsenic poisoning determines the maximum concentration of arsenic that it may contain. Of the two plants, Sudan grass is potentially more deleterious to humans or livestock than bean since it can grow in higher concentrations of arsenic and thus contain higher concentrations.

Distribution of arsenic within the plants

The concentrations of arsenic in the parts of the Sudan grass plants at the time of each collection over the range of 1 to 10 p.p.m. are plotted in figures 2 to 5 from data for series I. Similar data for series II repeat the initial portions of the curves of figures 2 to 5. The leaves and nodes contain the highest concentrations, the internodes are intermediate but below the plant top averages; the inflorescences are very low. These differences become more marked with time in series I because of a tendency for the concentration of arsenic in the nodes and leaves to increase, although those of the tillers and internodes show no change. These increases are not reflected in the curves for the plant tops (fig. 1) because they are offset by the appear-

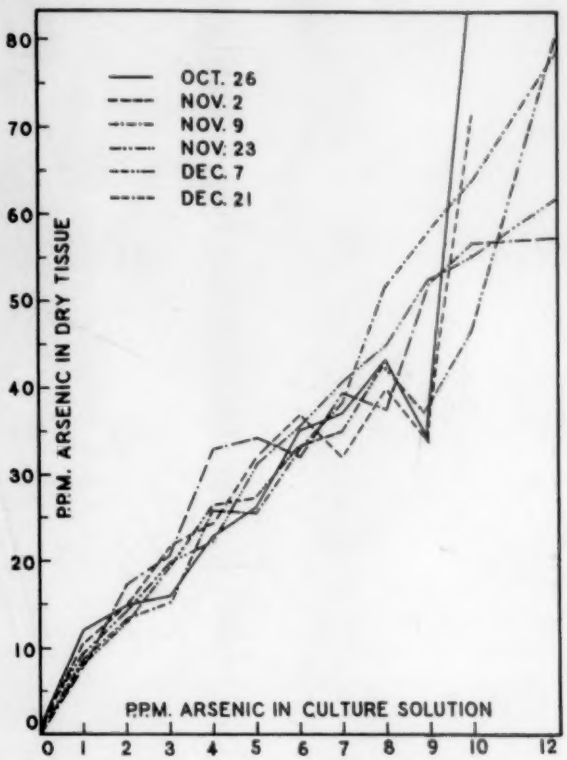
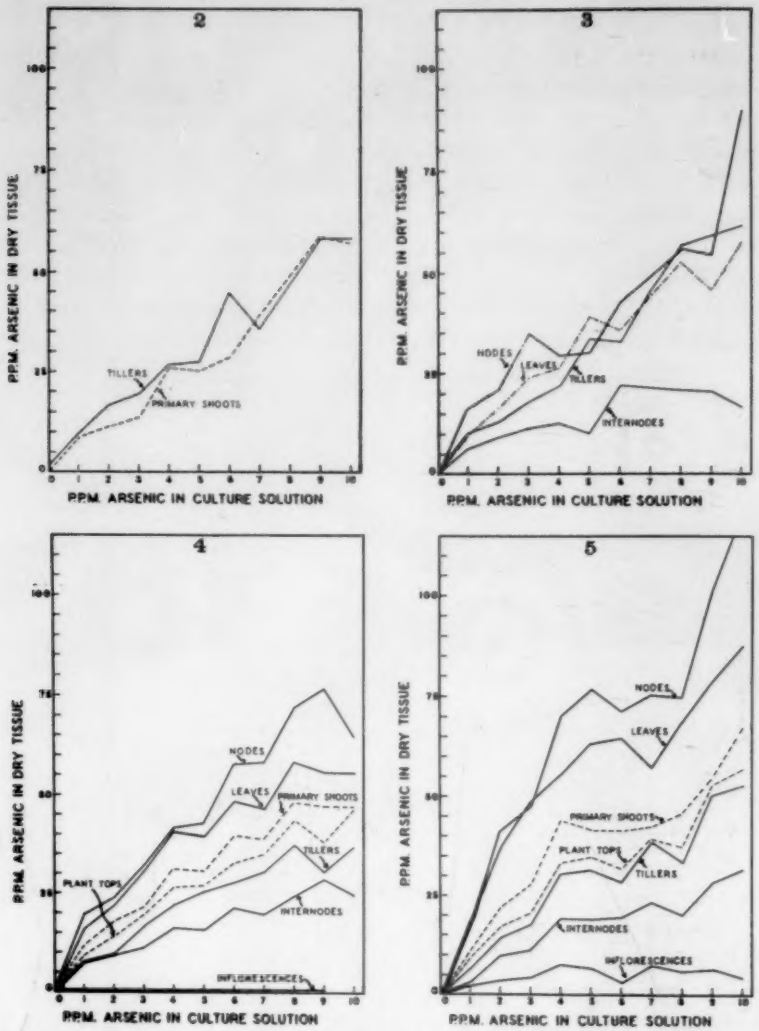


Fig. 1. Concentrations of arsenic in the plant tops of Sudan grass after growing for different lengths of time in culture solutions containing various concentrations of arsenic. The arsenic treatment began on Oct. 19, 1938.

ance of the low-concentration inflorescences and because, as the plants aged, the leaves came to represent a relatively smaller portion of the plant top. The close agreement of the curves for the plant tops, primary shoots, and tillers was to be expected (figs. 5, 6) for each represents an average of the



FIGS. 2–5. Concentrations of arsenic in different parts of Sudan grass after growing for 3 weeks (fig. 2), 5 weeks (fig. 3), 7 weeks (fig. 4), and 9 weeks (fig. 5) in culture solutions containing various concentrations of arsenic.

concentrations of arsenic in the nodes, leaves, internodes, and inflorescences.

The distribution in the bean plant is similar (fig. 6). The leaves are highest, stems and petioles intermediate, and the fruit lowest in concentration of arsenic. Except for the reproductive structures the concentration of arsenic in any specific part of either plant species is a function of the solution concentration.

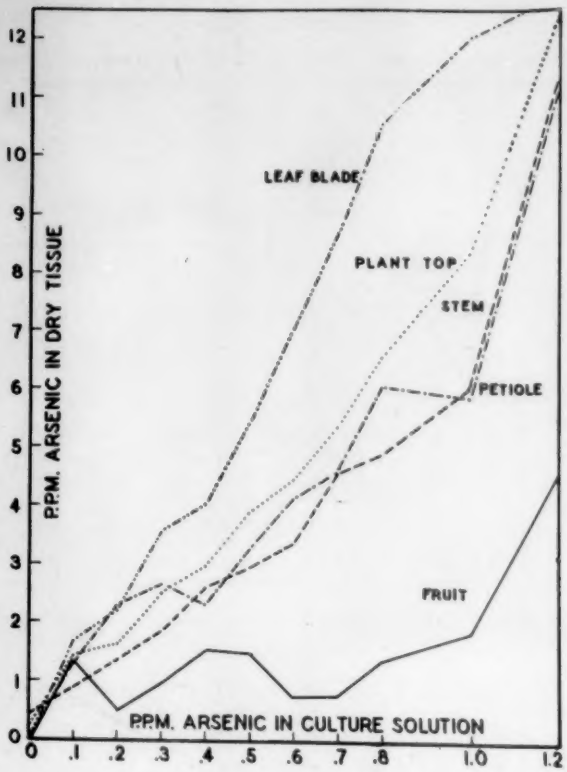


FIG. 6. Concentrations of arsenic in different parts of bean plants grown for 29 days in culture solutions containing various concentrations of arsenic.

The percentage of the total arsenic of the primary shoot of a plant found in any one part of the plant, *i.e.*, the percentage of the total arsenic in the leaves, for example, was the same for all the plants irrespective of the arsenic concentration in the solution. Similarly, the dry weight of each part of the primary shoot relative to the total dry weight of the primary shoot did not vary with the solution concentrations. Averages and standard deviations of these measures are presented in table III. These calculations are based on the Sudan grass and bean plants growing in concentrations of arsenic up to and including 10 and 1.2 p.p.m. As, respectively. Similar calculations made from data from plants growing in concentrations in excess of the foregoing deviated considerably from the averages, a result attributed to the non-functioning root systems of these plants.

The changes with time of the distribution of the weight of Sudan grass are in accordance with expectations based on the nature of the growth of these plants. The decrease in the relative weight of the leaves of the

TABLE III

DISTRIBUTION OF THE TOTAL ARSENIC AND DRY WEIGHT OF SUDAN GRASS AND BEAN PLANTS AMONG THEIR SEVERAL PARTS. EXPLANATION IN TEXT

	DRY WEIGHT OF THE PLANT PART AS A PERCENTAGE OF THAT OF THE PRIMARY SHOOT								DRY WEIGHT OF PRIMARY SHOOT AS A PERCENTAGE OF THAT OF THE PLANT TOP	
	LEAF		NODE		INTERNODE		INFLORESCENCE			
	%	S.D.*	%	S.D.	%	S.D.	%	S.D.	%	S.D.
Sudan grass series I, Dec. 7	53.8	7.1	8.5	2.7	35.5	4.5	3.2	2.3	45.5	8.1
Sudan grass series I, Dec. 21	41.5	4.8	9.3	0.4	37.1	8.3	11.8	2.3	34.5	5.1
Sudan grass series II, Dec. 9	54.5	3.9	9.2	1.4	35.2	2.8	2.1	1.4	62.8	6.3
Sudan grass series II, Dec. 25	38.4	2.3	8.8 (Petiole)	0.8	39.9 (Stem)	2.0	13.1 (Fruit)	2.4	37.3	6.2
Bean series II	49.2	5.7	7.1	0.4	20.3	2.3	23.5	1.9	.....	.....
	ARSENIC CONTENT OF THE PLANT PART AS A PERCENTAGE OF THAT OF THE PRIMARY SHOOT								ARSENIC CONTENT OF PRIMARY SHOOT AS A PERCENTAGE OF THAT OF THE PLANT TOP	
Sudan grass series I, Dec. 7	68.7	5.3	12.5	9.0	18.8	3.7	.....	.....	53.4	7.9
Sudan grass series I, Dec. 21	65.8	1.8	16.0	1.4	16.6	1.3	1.7	0.5	42.3	4.9
Sudan grass series II, Dec. 9	73.0	4.4	10.6	2.7	16.6	3.2	.....	.....	73.6	6.4
Sudan grass series II, Dec. 25	66.9	8.1	9.5 (Petiole)	1.7	22.6 (Stem)	4.1	3.3 (Fruit)	1.7	39.9	8.5
Bean series II	69.2	8.3	6.5	1.7	16.3	3.7	8.0	6.8	.....	.....

* S.D. = standard deviation.

primary shoots is a reflection of the fact that during the last two weeks of growth of both series the primary shoots were entering the reproductive phase of activity and hence were not producing any new leaf or stem tissue although the tillers continued to do so. That the concentrations of arsenic in the solutions or in the plants did not affect these distributions indicates that the arsenic suppressed the growth of the entire plant and not of any paricular morphological part. This suggests an interference with the functioning of some one or more of the fundamental processes of the plants.

The distribution of arsenic is unaffected by either the period of growth in the solutions or the concentrations of arsenic in the solutions. Although both the nodes and leaves were previously observed to contain nearly the same concentrations of arsenic it may be seen in the table that approximately two-thirds of all the arsenic entering the primary shoots of either

Sudan grass or bean is carried to the leaves whereas the nodes contain a relatively small portion of the total arsenic.

The high concentrations of arsenic in the leaves and nodes are of interest because neither of these parts show any macroscopic signs of injury other than the initial desiccation of the leaf tips. When the nodes were split longitudinally for drying, both the nodal plate and nodal meristem were observed to be white and apparently healthy. Apparently the plants can in some manner render the arsenic inactive. The fact that the arsenic exerted a strong toxic action on the roots, which was expressed in one form as a discoloration, is not inconsistent with the above suggestion; after a period as short as one week in the solutions, relatively enormous concentrations of arsenic were found associated with the roots of Sudan grass. Thus, when the solution concentrations were 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16 and 18 p.p.m. of As, the concentrations in or on the roots as p.p.m. As of dry weight were 338, 263, 390, 514, 655, 734, 903, 906, 1026, 1094, 1246, 1386, 1059 and 1461. These concentrations are several times greater than the highest observed values in the leaves or nodes.

Significance of the arsenic contents of the plants

The magnitude of the concentrations of arsenic found in the plants is of importance both in relation to the physiology of the plant and with respect to the health of humans and livestock that could ingest such plants as food. A complete evaluation of their significance with reference to the latter point would require the consideration of at least the following factors: (1), the concentration of arsenic in the plant and in each of its parts; (2), the chemical state of the arsenic; (3), the magnitudes of the quantities of arsenic that may be considered deleterious to mammals; and (4), the potentiality for growth of plants that contain these magnitudes of arsenic.

The Federal Food and Drugs Act limits the quantity of arsenic that may be present in foods as an adulterant to 0.01 grains per pound which is equal to 1.08 p.p.m. of As. Foods containing more than this content are subject to seizure. According to law none of the arsenic present in the plants being considered here is present as an adulterant. This limit does not mean that quantities of arsenic less than 1.08 p.p.m. are of no concern. Meyers *et al.* (**19**) have on several occasions pointed out the injurious effects of small amounts of arsenic in their relation to the public health.

It is probable that all or part of the arsenic in the plants is tied up in organic molecules which may well affect its toxicity to mammals. A statement by White (**28**) of the U. S. Food and Drug Administration, made in relation to the natural arsenic content of marine products, is relevant: "The data are given with the full recognition that the arsenic may (in the marine products) in some cases, be present in organic combinations which render it

TABLE IV

CONCENTRATIONS OF ARSENIC IN DIFFERENT PARTS OF SUDAN GRASS WHEN GROWN IN CULTURE SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF ARSENIC. DATA FROM THE DEC. 21 COLLECTION OF SUDAN GRASS SERIES I

Culture concentration	Leaves		Nodes	Internodes	Inflorescences	Tillers		Primary shoots		Plant tops		Yield
	Dry weight	Fresh weight*	Dry weight	Dry weight	Dry weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Percentage
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	%
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
1	*18.9*†	*2.7*	*17.1*	*3.4*	*2.1*	*6.6*	*1.1*	*10.5*	*2.5*	*8.1*	*1.5*	74
2	*36.6*	*5.2*	*41.1*	*9.9*	*3.6*	*14.0*	*2.5*	*22.0*	*5.3*	*17.2*	*3.4*	55
3	*48.0*	*6.9*	*47.3*	*11.2*	*4.6*	*17.8*	*3.0*	*27.9*	*6.7*	*20.7*	*3.9*	48
4	*55.3*	*7.9*	*69.8*	*19.1*	*7.8*	*30.4*	*5.2*	*44.0*	*7.2*	*33.0*	*6.6*	35
5	*63.5*	*9.1*	*76.7*	*19.0*	*6.8*	*31.3*	*4.7*	*41.8*	*8.1*	*34.7*	*5.6*	40
6	*64.8*	*9.3*	*71.3*	*19.5*	*3.3*	*28.3*	*4.5*	*41.6*	*8.8*	*32.0*	*5.4*	35
7	*56.9*	*8.1*	*75.4*	*23.2*	*7.5*	*38.4*	*5.7*	*42.3*	*8.6*	*39.4*	*6.4*	22
8	*68.3*	*9.7*	*74.9*	*20.2*	*6.0*	*33.4*	*5.2*	*44.9*	*9.2*	*37.5*	*6.4*	28
9	*78.8*	*11.3*	*101.2*	*28.1*	*6.4*	*50.0*	*8.2*	*54.9*	*11.9*	*52.3*	*9.7*	21
10	*87.4*	*12.5*	*126.8*	*31.2*	*4.5*	*52.8*	*8.9*	*67.2*	*14.6*	*56.9*	*10.3*	22

* The fresh weight of the leaves was not determined. These values are estimated from an assumed moisture content of 85.7 per cent.
† Values italicized are in excess of the tolerance limit.

TABLE V

CONCENTRATIONS OF ARSENIC IN DIFFERENT PARTS OF SUDAN GRASS WHEN GROWN IN CULTURE SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF ARSENIC. DATA FROM THE DEC. 9 COLLECTION OF SUDAN GRASS SERIES II

CULTURE CONCENTRATION	LEAVES		NODES	INTERNODES	TILLERS		PRIMARY SHOOTS		PLANT TOPS		YIELD
	DRY WEIGHT	FRESH WEIGHT*	DRY WEIGHT	DRY WEIGHT	DRY WEIGHT	FRESH WEIGHT	DRY WEIGHT	FRESH WEIGHT	DRY WEIGHT	FRESH WEIGHT	PERCENTAGE
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	%
0.00	0.7	0.1	0.0	0.0	0.0	0.0	0.4	0.1	0.3	0.0	100
0.25	*7.2*†	1.0	*3.5*	*3.0*	*2.2*	0.3	*5.2*	0.8	*3.9*	0.6	110
0.50	*7.5*	*1.1*	*5.1*	*2.5*	*3.6*	0.4	*5.6*	0.8	*4.7*	0.6	100
0.75	*10.3*	*1.5*	*12.7*	*4.6*	*5.4*	0.6	*8.3*	*1.2*	*7.4*	1.0	95
1.00	*13.7*	*2.0*	*11.8*	*4.1*	*5.5*	0.6	*9.3*	*1.5*	*7.8*	*1.1*	88
1.25	*19.6*	*2.8*	*14.1*	*4.7*	*8.1*	0.9	*13.8*	*2.0*	*11.2*	*1.5*	77
1.50	*18.4*	*2.6*	*16.5*	*7.5*	*8.7*	1.0	*14.5*	*2.1*	*12.4*	*1.7*	88
1.75	*20.7*	*3.0*	*20.2*	*8.7*	*10.7*	*1.3*	*16.4*	*2.4*	*14.2*	*1.9*	83
2.00	*24.9*	*3.6*	*20.2*	*7.7*	*11.5*	*1.4*	*17.8*	*3.4*	*15.6*	*2.4*	85
2.50	*27.8*	*4.0*	*29.7*	*10.5*	*16.4*	*1.9*	*22.4*	*3.3*	*20.4*	*2.8*	75

* The fresh weight of the leaves was not determined. These values are estimated from an assumed moisture content of 85.7 per cent.
† Values italicized are in excess of tolerance limit.

TABLE VI

CONCENTRATIONS OF ARSENIC IN DIFFERENT PARTS OF BEAN WHEN GROWN IN CULTURE SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF ARSENIC. DATA FROM BEAN SERIES II

Culture concentration	Leaves		Petioles		Stems		Fruit		Plant tops		Yield
	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Percentage
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	%
0.0	0.37	0.07	0.12	0.02	0.48	0.10	0.07	0.01	0.32	0.05	100
0.1	*1.66**	0.28	*1.29*	0.15	0.87	0.15	*1.30*	0.12	*1.38*	0.19	112
0.2	*2.17*	0.37	*2.20*	0.25	*1.34*	0.23	0.48	0.04	*1.60*	0.22	92
0.3	*3.56*	0.60	*2.61*	0.26	*1.87*	0.31	0.91	0.08	*2.48*	0.33	114
0.4	*4.00*	0.62	*2.37*	0.26	*3.74*	0.66	0.74	0.08	*3.88*	0.39	131
0.5	*5.39*	0.89	*3.23*	0.33	*2.92*	0.49	*1.47*	0.13	*4.43*	0.43	95
0.6	*7.06*	*1.13*	*4.10*	0.42	*3.33*	0.58	0.73	0.07	*5.36*	0.59	100
0.7	*8.68*	*1.47*	*4.58*	0.46	*4.50*	0.77	0.75	0.07	*6.52*	0.70	68
0.8	*10.56*	*1.91*	*6.01*	0.61	*4.87*	0.84	*1.32*	0.12	*8.35*	0.87	52
1.0	*12.07*	*2.57*	*5.83*	0.60	*6.04*	0.88	*1.80*	0.16	*12.33*	*1.20*	45
1.2	*13.66*	*3.98*	*11.04*	*1.24*	*11.06*	*1.37*	*4.45*	0.70	*12.06*	*2.39*	15

* Values italicized are in excess of the tolerance limit.

somewhat less toxic than when in the form of lead, calcium, or other arsenates. It is doubtful, however, whether any toxicologist would assert that there is any published work to prove that the natural arsenic in marine and other foods is without any harmful effect." This statement indicates that even the smallest quantities of arsenic in the plants are significant. The same reasoning applies to the effects of arsenic on livestock and to the rôle of arsenic in meat food products.

For the purpose of this report the tolerance limit of 1.08 p.p.m. of As will be used as a test of significance. The arsenic concentrations in bean and Sudan grass on the fresh and dry weight bases for representative collections of plants are tabulated in tables IV, V, VI, and VII. Values italicized are in excess of the tolerance limit. These data show that:

TABLE VII

CONCENTRATIONS OF ARSENIC IN THE PLANT TOPS OF BEAN WHEN GROWN IN CULTURE SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF ARSENIC. DATA FROM BEAN SERIES I

CULTURE CONCENTRATION	DRY WEIGHT	FRESH WEIGHT	YIELD
p.p.m.	*p.p.m.*	*p.p.m.*	%
0.00	*2.5*†	0.3	100
0.25	*3.6*	0.5	58
0.50	5.2	0.7	42
0.75	*3.9*	0.5	26
1.00	*4.3*	0.6	20
2.00*	*17.9**	*2.4**	4*
3.00*	*27.3**	*3.7**	−6*
4.00*	*32.2**	*4.4**	−1*
5.00*	*35.7**	*4.8**	0*
6.00*	*72.9**	*9.8**	−7*
7.00*	*99.5**	*13.4**	−3*
8.00*	*91.5**	*12.4**	−2*
9.00*	*100.6**	*13.6**	6*
10.00*	*110.9**	*15.0**	6*

* These plants were immediately killed upon the addition of arsenic to the solutions as evidenced by the fact that they made no growth whatsoever. The negative values are caused by variation in the weights of the seedlings at the beginning of the treatment.

† Values italicized are in excess of the tolerance limit.

1. On the dry weight basis, all of the plants and each of their parts exceeded the limit with the exception of the fruit and stem of bean. Particularly significant are the concentrations in those plants whose *yield was not lowered* by the arsenic.

2. On the fresh weight basis, only the leaves of plants whose *yield was unaffected* by the arsenic exceeded the tolerance limit. Sudan grass plants whose yields were slightly decreased exceeded the tolerance limit in all categories whereas a similar result obtains with bean only with an almost complete suppression of growth.

These results follow logically from the interaction of previously observed factors: (1), arsenic is accumulated in the plant tops in concentrations determined primarily by the concentrations in the nutrient solutions, irrespective of the plant species; and (2), the toxicity of the arsenic to the plants is a function of the particular plant species, Sudan grass being some 8 to 10 times more resistant than bean. Thus the more resistant species (Sudan grass) is able to grow under conditions conducive to higher concentrations of arsenic within itself than is the more sensitive species (bean). For equal relative reductions in yield the more resistant species contains several times the concentration of arsenic in the sensitive species. Furthermore, the leaves contain markedly higher concentrations of arsenic than the plant top as a whole, a factor of significance if they are to be used alone as food.

Discussion

In the introduction it was suggested that the gradual accumulation of arsenic in soils as a result of the extensive use of arsenicals for the control of insects and weeds was a possible source of arsenic in foods through its absorption by the plant. The data presented have demonstrated that exceedingly minute quantities of arsenic in the nutrient medium give rise to concentrations of arsenic in the plant tissues that cannot be ignored. Although the toxicity of arsenic and its accumulation from a soil medium may differ substantially in both magnitude and nature from that observed here, arsenic in the soil must be considered as a source from which it can be absorbed in significant quantities by plants, until otherwise demonstrated. Certain combinations of plant, soil, and arsenical would seem to be particularly conducive to the development of high arsenic content plants.

The two classes of arsenicals in general use today are the insoluble arsenates as insecticides and the soluble arsenites as herbicides. They differ in their effects on plants and in their histories in the soil.

Pentavalent arsenic is decidedly less toxic than trivalent arsenic (**3, 4, 18**). Whereas 10 p.p.m. of As in the form of sodium arsenite are lethal to tomato, the same plant can withstand over three times this concentration of arsenic in the form of sodium arsenate (**15**). Absorption may thus take place over a corespondingly wider range of concentrations of pentavalent arsenic than trivalent.

In the soil, absorption of either form is restricted to the available arsenic which is presumably that in solution but may include part of the insoluble or fixed arsenic. ROSENFELS and CRAFTS (**23**) have obtained data suggesting that part of the insoluble or fixed arsenic may be available to the plant. The available arsenic bears no direct relationship to the total arsenic of a soil because soils vary widely in the rate and degree of fixation of arsenic. The effect of the composition of the soil on the concentration of available arsenic

as measured by its toxicity to biological indicators has been studied for arsenites by CRAFTS and his co-workers (**8, 9, 10, 23**), and for arsenates by GILE (**13**), STEWART and SMITH (**24**), PADEN and his associates (**7, 21**), and others. These studies indicate that, in general, the heavier the soil the greater is its ability to fix the arsenic. Conversely, leaching of arsenic tends to be more rapid from lighter soils. An exception is the ferruginous soil type which has a very high fixation capacity for arsenic.

When insoluble arsenates are added to the soil they are, of course, at first unavailable to the plant. Under the action of the soil solution, however, they are partially dissolved (**24**). As the total arsenic of the soil is increased by annual additions of the arsenical, the available fraction should increase. Because of their low fixation capacity, light soils would tend to become toxic with fewer applications of the arsenical than would heavy soils. It is the lighter soils that have become unproductive in South Carolina (**1**) as a result of annual applications of calcium arsenate in the control of the cotton boll weevil. Leaching must obviously have taken place at a slower rate than had accumulation in this soil. Numerous soil analyses indicate that most of the added arsenicals are retained in the upper few inches (**14, 17, 27**). These insoluble arsenicals thus constitute a long-time reserve which, under the influence of the soil solution, is slowly made available to the plant.

The soluble arsenicals, upon addition to the soil, are immediately available in high concentrations. More or less of the arsenical is then fixed to an extent and at a rate determined by the soil type. Two possible undesirable consequences may result from the use of herbicides: (1) an unusually heavy application may so increase the available arsenic content of the soil for a short time as to allow appreciable absorption and perhaps cause injury to the plants; (2) annual applications can build up a reserve of fixed arsenic which will function similarly to the insoluble arsenicals. If the fixation capacity of the soil is exhausted, then further application would be very potent until leached away.

What appears to be an example of injury from the unrestricted use of a soluble arsenical was observed during the course of this investigation. An herbicide was applied to the underbrush of an orchard of macadamia nut trees at Honokaa, Hawaii. Sometime later some of the trees became sickly. It was noted that many of the roots of the trees were either exposed or very near to the surface of the soil. Samples of the roots of diseased and adjacent healthy trees were obtained by first cutting away the surface layers. These and samples of the leaves were analyzed for arsenic with results as shown in table VIII. The roots of the diseased trees contained much higher concentrations of arsenic than did those of adjacent healthy ones.

Because arsenicals tend to accumulate in the upper layer of the soil,

TABLE VIII

CONCENTRATIONS OF ARSENIC (AS As_2O_3) IN THE ROOTS AND LEAVES OF DISEASED AND HEALTHY MACADAMIA NUT TREES*

DISEASED TREES			ADJACENT HEALTHY TREES		
TREE NO.	ROOTS	LEAVES	TREE NO.	ROOTS	LEAVES
	p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*
A–41–13	18.8	8.2	A–41–14	9.3	7.3
A–16–31	29.2	24.1	A–16–32	1.3	5.7
A–10–13	11.5	6.4	A–40–12	6.4	6.3

* The samples were collected and the observations made by the Department of Soil Chemistry, University of Hawaii Experiment Station.

shallow-rooted plants are most likely to accumulate arsenic, as well as to be injured by it.

Finally, plants that are relatively resistant to arsenic poisoning are those capable of growing successfully and, at the same time, of accumulating excessive quantities of arsenic. Such plants would give no indication of their arsenic contents if they responded in a manner similar to bean and Sudan grass in nutrient cultures. It also follows from this investigation that plants which are predominantly leafy, such as spinach and lettuce, may attain very high concentrations of arsenic.

Summary

When Sudan grass (*Sorghum vulgare* var. *sudanense*) and bush bean (*Phaseolus vulgaris* var. *humilis*) were grown for varying lengths of time in nutrient solutions containing various concentrations of $NaAsO_2$, it was found that concentrations of arsenic up to 0.5 to 0.6 p.p.m. had no effect on the growth of either plant as measured by increases in dry weight. Growth was effectively suppressed, however, by concentrations of 1.2 and 12.0 p.p.m. of As for bean and Sudan grass, respectively. Addition of arsenic to the solutions caused an immediate plasmolysis of the roots and wilting of the leaves followed by discoloration of roots and necrosis of leaf tips and margins, indicating that the arsenic effected a sudden decrease in the movement of water into the plants.

The concentrations of arsenic in the plant tops and in each of their parts except the reproductive structures were found to be proportional to the concentrations in the nutrient solutions. The magnitudes of these concentrations were more or less constant during the experimental periods, the longest of which was nine weeks. The leaves were highest, the stem tissues intermediate, and the reproductive structures lowest in concentration of arsenic.

Within a narrow range of concentrations of arsenic in the nutrient medium both plants grew successfully; but they contained concentrations of arsenic in their plant tops in excess of the tolerance limit (1.08 p.p.m. of As)

established by law for the arsenic content of foods. For equal relative reductions in yield the more resistant species (Sudan grass) contained several times the concentration of arsenic in the sensitive species (bean).

Accumulated arsenical residues in soils as a result of insect and weed control practices are discussed as a source of arsenic contamination in foods through absorption by plants.

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LITERATURE CITED

1. ALBERT, W. B., and PADEN, W. R. Calcium arsenate and unproductiveness in certain soils. Science n.s. **73**: 622. 1931.
2. ARNON, D. I., and STOUT, P. R. The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiol. **14**: 371–375. 1939.
3. BRENCHLEY, W. E. On the action of certain compounds of zinc, arsenic, and boron on the growth of plants. Ann. Bot. **28**: 283–301. 1914.
4. BROOKS, M. M. Studies on the permeability of living and dead cells. Public Health Reports **38**: 2074–2086; 2951–2966. 1923.
5. CASSIL, C. C. Report on arsenic. Jour. Assoc. Off. Agr. Chem. **20**: 171–177. 1937.
6. ————, and WICHMANN, H. J. A rapid volumetric method for determining arsenic. Jour. Assoc. Off. Agr. Chem. **22**: 436–445. 1939.
7. COOPER, H. P., PADEN, W. R., HALL, E. E., ALBERT, W. B., ROGER, W. B., and RILEY, J. A. Effect of calcium arsenate on the productivity of certain soil types. South Carolina Exp. Sta. 44th Ann. Rept. 28–36. 1931.
8. CRAFTS, A. S. The toxicity of sodium arsenite and sodium chlorate in four California soils. Hilgardia **9**: 461–498. 1935.
9. ————. The relation of nutrients to toxicity of arsenic, borax, and chlorate in soils. Jour. Agr. Res. **58**: 637–671. 1939.
10. ————. Toxicity studies with arsenic in eighty California soils. Hilgardia **12**: 177–200. 1939.
11. FELLENBERG, T. Über den Arsengehalt natürlicher und mit Arsenpra-

paraten behandelter Lebensmittel. Biochem. Zeitschr. **218**: 300–317. 1930.

12. FREE, E. E. Symptoms of poisoning by elements in Pelargonium and other plants. Johns Hopkins Univ. Circ. n.s. no. **3**: 197–198. 1917.
13. GILE, J. E. Toxicity of calcium arsenate to millet. Jour. Agr. Res. **52**: 477–491. 1936.
14. HEADDEN, W. P. Arsenical poisoning of fruit trees. Colorado Agr. Exp. Sta. Bull. 131. 1908.
15. HEGGENESS, H. Thesis. University of Hawaii. 1940.
16. JADIN, F., and ASTRUC, A. L'Arsenic et la manganèse dans les feuilles jeunes et agés. Compt. Rend. Acad. Sci. (Paris) **156**: 2023–2024. 1913, and **159**: 268–270. 1914.
17. JONES, J. S., and HATCH, M. B. The significance of inorganic spray residue accumulations in orchard soils. Soil Sci. **44**: 37–63. 1937.
18. MORRIS, H. E., and SWINGLE, D. B. Injury to growing crops caused by the application of arsenical compounds to soil. Jour. Agr. Res. **34**: 59–78. 1927.
19. MYERS, C. N., THRONE, B., GUSTAFSON, F., and KINGSBURY, J. Significance and danger of spray residue. Ind. & Eng. Chem. **25**: 624–628. 1933.
20. Official and tentative methods of analysis of the A.O.A.C. 1935.
21. PADEN, W. R., and ALBERT, W. B. Effect of the addition of arsenical compounds to the soil. South Carolina Exp. Sta. 43rd Ann. Rept. p. 32. 1930
22. REED, J. F., and STURGIS, M. B. Toxicity from arsenic compounds to rice on flooded soils. Jour. Amer. Soc. Agron. **28**: 432–436. 1936.
23. ROSENFELS, R. S., and CRAFTS, A. S. Arsenic fixation in relation to the sterilization of soils with sodium arsenite. Hilgardia **12**: 203–229. 1939.
24. STEWART, J., and SMITH, E. X. Some relations of arsenic to plant growth. I and II. Soil Sci. **14**: 111–125. 1922.
25. STOKLASSA, J. De l'importance physiologique due manganèse et de l'aluminium dans la cellule végétale. Compt. Rend. Acad. Sci. (Paris) **152**: 1340–1342. 1911.
26. STOCKBERGER, W. W. The sources of arsenic in certain samples of dried hops. U. S. D. A. Bur. Plant Ind. Bull. 121. pp. 41–46. 1908.
27. VANDECAVEYE, S. C., HORNER, B. M., and KEATON, C. K. Unproductiveness of certain orchard soils as related to lead arsenate spray accumulations. Soil Sci. **42**: 203–215. 1936.
28. WHITE, W. B. Poisonous spray residues on vegetables. Ind. & Eng. Chem. **25**: 621–623. 1933.

WATER UPTAKE AND ROOT GROWTH AS INFLUENCED BY INEQUALITIES IN THE CONCENTRATION OF THE SUBSTRATE

FRANK M. EATON

(WITH FIVE FIGURES)

Introduction

When a number of solutions of unlike concentration are presented at one and the same time either to an established or to a developing root system, questions follow as to whether water is withdrawn as rapidly from the concentrated as from the dilute regions and as to whether new roots extend themselves as readily in the regions of high salinity as in those of low salinity. The subject is of agronomic, as well as physiological, significance since the productivity of soils must be regarded as being contingent not only on their nutrient and water storage resources but also upon the readiness with which these resources can be utilized by plants.

A substantial diversity is customarily found in the salt concentrations of the soil solution within the root zone of single plants growing on irrigated lands. The data of table I, which are presented as an illustration, represent

TABLE I

VARIABILITY OF CHLORIDE, SULPHATE, AND NITRATE CONCENTRATIONS (MILLEQUIVALENTS PER LITER) IN DISPLACED SOIL SOLUTIONS OF A WELL-DRAINED BUT SPARINGLY IRRIGATED ORANGE ORCHARD IN THE SIERRA MADRE FOOTHILL AREA OF SOUTHERN CALIFORNIA. SAMPLES WERE COLLECTED ON THE NORTHWEST AND NORTHEAST SIDE OF EACH OF TWO TREES

DEPTH	TREE 30, ROW 22						TREE 36, ROW 22					
	SO_4		Cl		NO_3		SO_4		Cl		NO_3	
	NW	NE	NW	NE	NW	NE	NW	NE	NW	NE	NW	NE
feet	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
0– 3	2.3	3.3	1.2	2.4	4.5	5.0	9.1	2.1	1.6	1.1	2.3	2.0
3– 6	11.8	36.6	1.8	25.2	0.9	4.8	53.3	40.3	13.1	12.1	9.6	8.1
6–10	17.2	40.4	4.2	81.6	4.1	10.5	43.5	42.6	47.9	16.5	25.7	10.2

analyses of displaced soil solutions from samples collected in a sparingly irrigated but relatively permeable and well drained orchard soil. Conductivity measurements made on the solutions displaced from the separate one-foot horizons before combining them for analyses showed a greater variability than is here indicated; the variability in more heavily irrigated soils, however, is usually lower than that found in this instance. All of the

samples were wetted to approximately the moisture equivalent before displacement.

The experimental work here reported upon includes a series of tests conducted in water cultures with tomato and corn plants. The root systems of these plants were divided between solutions that differed in concentration and in the character of the added salts. The effects of the treatments were measured in terms of root growth and water uptake.

Review of literature

The review of literature that follows is confined to papers concerned primarily with the cross transfer of water and solutes in plants. The problem under consideration, however, bears also upon broader fields of inquiry that concern some of the forces involved with water uptake and with some of those governing the growth of roots.

GILE and CARRERO (**8**) found the total amount of nitrogen or phosphorus absorbed by rice plants having half of their roots in complete nutrient solution and half in solutions deficient in these elements was 76 per cent. of that of plants having all roots supplied with complete nutrient solution. In a subsequent paper (**9**) they reported a lesser growth of the portion of the root system of corn plants which extended into solutions deficient in nitrogen, phosphorus, or potassium. Their solutions were all dilute (about 5 m.e. of total bases per liter), so there could have been but little difference in osmotic concentrations. MCMURTREY (**14**) using a similar technique, reported an unilateral development of the leaves of tobacco plants with roots divided between complete nutrient and element-deficient nutrient solutions. He mentioned, and one of his photographs shows, increased root growth when calcium (previously omitted) was added to one of the two solutions supplied to a single plant.

AUCHTER (**1**) applied nitrate of soda on one side of field trees and concluded that under normal conditions mineral nutrients absorbed by roots on one side are translocated to and used by the trunk, limbs, and leaves above them and that foods manufactured by one side are used or stored on that side or translocated to the roots of the same side. The behavior of trees with half of their roots severed prompted the conclusion that water can move laterally in trees without much difficulty. FURR (**7**), finding the volume of lemon fruits to be independent of position over cut and uncut roots, also concluded that there was a ready cross-transfer of water throughout old lemon trees. AUCHTER and SCHRADER (**2**), in experiments continued for two years, thought there was little if any cross transfer of carbohydrates from sun-exposed to cloth-enclosed sides of apple trees. MILLER (**15**), has reviewed a series of papers showing a tendency toward increased root development in fertilized portions of soils. CALDWELL (**4**) reports an asymmetric develop-

ment of the fleshy root of the Swede turnip when leaves were removed from one side. The roots below the defoliated side of Coleus plants (5) grew less than the roots under the foliated side. Experiments (1, 5) on plants with roots divided between rich soil and sand have added support to the foregoing conclusions. NIXON[1] has found that date fruits on strands subtended below, and in line with, a notch cut in the fruit stalk that severed about 50 per cent. of the vascular system increased in dry matter during the summer; this indicates some cross-transfer of manufactured foods from the unsevered to the severed side of the stalk.

Methods

Both corn and tomato plants were used for the experiments. The former was regarded a nearly ideal test plant since its vascularity is such as to largely preclude a unilateral development of the top or a differential accumulation of salt in the leaves of one side. It will be recalled that each leaf sheath of the corn plant encircles the stalk at the node from which it arises and that, owing to anastomoses, the nodal structures favor lateral distribution of constituents rising or descending through the vascular system. Anatomical studies of the vascular system of the potato would imply that some lateral distribution of solutes rising or descending in tomato shoots is likewise to be expected.

Lacquered rectangular metal cans with openings cut in one corner, or in the middle of one side of the top, large enough to admit a group of roots were used as culture vessels. These containers (figure 5) used in groups of two or four, were bound together in such a manner that portions of the root system of a single plant could be extended into each. Water was added to the cans through openings having screw caps. Gallon containers were used in experiments 1 and 2, and quart containers in the other experiments.

The plants were grown to a height of 15 to 25 cm. in nutrient solutions in preparation for experimental use and the separation of roots into groups. After dividing the roots into 2 or 4 equal groups, trimming when necessary, they were dusted heavily with carbon black to facilitate a differentiation at the end of the experimental period between the original roots and those subsequently developed.

The plants were fastened in place with cork and cotton mountings, and the roots at the point of division were separated by cork wedges 1 cm. tall that rested on the edges of the cans. Further, to make unlikely the movement of solution from one container to another by wick action, the levels of the solutions were not brought nearer than 1.5 cm. to the tops of the containers. A can with a similar opening was set up in conjunction with each experiment to measure direct evaporation. The loss from this container has been deducted from all results to give net water-uptake values.

[1] R. W. NIXON, unpublished data.

The base nutrient solution contained calcium nitrate, potassium nitrate, magnesium sulphate and potassium dihydrogen phosphate in concentrations of 5, 5, 2 and 1 millimoles per liter respectively (HOAGLAND's solution). This solution was used in the foregoing concentration and in fractional and multiple concentration both alone and in conjunction with added chloride and sulphate salts. These latter salts were added 50 per cent. as m.e. of sodium, 25 per cent. as magnesium, and 25 per cent. as calcium. All solutions contained, in addition to the foregoing, 1 p.p.m. of boron, 0.2 p.p.m. of zinc, and 0.5 p.p.m. of manganese. Iron was added in the form of tartrate or citrate as needed.

The freezing point depressions, Δ° C, and electrical conductance, $K \times 10^{-5}$, and other determinations of concentration on used solutions were always made after the containers had been restored to their initial weights by adding distilled water. Distilled water was added frequently throughout the experiments to replace transpiration losses. All of the experiments were conducted in a greenhouse. Only the fresh weights of roots are reported since the concentration of the solutions did not affect significantly the percentage of moisture in the roots in those instances (experiment 3) when both fresh and dry weights were measured.

Results

EFFECT OF UNILATERALLY SUPPLIED SALTS ON ROOT GROWTH AND WATER UPTAKE. EXPERIMENTS 1 TO 7

In experiments 1 to 7, inclusive, the root systems of corn and tomato plants were divided between two or more solutions that differed in composition and concentration. The results of these experiments (tables II to V) are alike in showing a greater water-uptake from, and a greater root growth in, the solutions of low concentration than in those of high concentration.

In experiment 1 with corn (table II) nearly twice as great a weight of roots developed in a solution with a trace of chloride as in a solution with 10 m.e.; little or no root growth resulted in solutions with 50 and 250 m.e. of chloride (fig. 1). The effect on water-uptake was also marked. The solution with 250 m.e. of chloride, if it were the only one supplied, would probably be too saline for the growth of corn plants. This relation would not hold for 50 m.e. of chloride since corn plants on such solutions under similar conditions (**6**) made 62 per cent. as much total growth as in control solutions without chloride. The roots in the chloride solutions of those experiments were heavier relative to the weight of the shoots than in the control solutions.

Chloride determinations (table II) on the solutions at the beginning and end of experiment 1 indicate some movement of chloride through the roots into the solution that started with a trace of chloride.

In experiment 2, with a tomato plant, a significant reduction in water

FIG. 1. The differential development of new roots of a corn plant 22 days after the initial roots had been divided between vessels containing in addition to the base nutrient solution: a, trace; b, 10 m.e.; c, 50 m.e.; and d, 250 m.e. of chloride. The roots on the plants at the start of the experiment were dusted with carbon black. Experiment 1.

uptake and a lesser development of roots resulted when the roots were supplied with 22.5 m.e. of chloride; the effects of 7.5 m.e. of chloride, however, were negligible (table I). In this as well as in subsequent experiments with tomatoes, the compact matting of the roots made it difficult to differentiate very accurately between the old and new ones.

TABLE II

ROOTS OF CORN AND TOMATO PLANTS DIVIDED BETWEEN FOUR SOLUTIONS WITH DIFFERENT CONCENTRATIONS OF CHLORIDE. EXPERIMENTS 1 AND 2

CONTAINER	SOLUTION DESIGNATION	$K \times 10^{-5}$ AT 25° C.		CHLORIDE PER LITER		NEW ROOTS		WATER UPTAKE	
		INITIAL	END	INITIAL	END				
				m.e.	*m.e.*	*gm.*	%	*ml.*	%
Experiment 1. Corn plant in base solution with added chloride. Duration 22 days									
A	Trace Cl	220	210	0.10	2.14	8.8	63	908	66
B	10 m.e. Cl	320	298	10.17	9.69	5.1	36	348	26
C	50 m.e. Cl	705	691	48.21	47.50	0.1	1	53	4
D	250 m.e. Cl	2542	2486	254.0	244.0	0.0	0	55	4
Experiment 2. Tomato plant in base solution with added chloride. Duration 22 days									
A	Trace Cl	214	166	0.05	0.11	8.1*	31	820	35
B	7.5 m.e. Cl	291	241	7.46	7.07	8.2*	31	777	33
C	22.5 m.e. Cl	443	392	22.27	21.22	5.4	21	540	23
D	75 m.e. Cl	937	891	73.56	71.64	4.4	17	215	9

* The new and old roots in these containers were so badly matted together that it was impossible to differentiate very closely between them.

All of the original solutions of experiment 2 contained 15 m.e. of nitrate and 6 m.e. of potassium per liter. Measurements made at the end of the test showed that the concentrations in containers A, B, C and D had been reduced to 10.7, 10.9, 11.6, and 13.8 m.e. of nitrate, and to 4.2, 4.7, 4.9, and 6.2 m.e. of potassium per liter, respectively. It is probable that the greater number of roots in the solutions of lower concentration had as much to do with the

TABLE III

CORN ROOTS DIVIDED BETWEEN 4 SOLUTIONS WITH THE BASE NUTRIENT AND DIFFERENT CONCENTRATIONS OF CHLORIDE. DURATION 28 DAYS. EXPERIMENT 3, DUPLICATE TESTS

CONTAINER	SOLUTION DESIGNATION	NEW SOLUTIONS		END SOLUTIONS $K \times 10^{-5}$ AT 25° C.	NEW ROOTS		WATER UPTAKE	
		Δ	$K \times 10^{-5}$ AT 25° C.					
		°C.			*gm.*	%	*ml.*	%
Plant no. 1								
A	Trace Cl	0.016	215	55.9	16.5	33	2291	39
B	10 m.e. Cl	0.091	317	158.0	14.7	29	1941	33
C	20 m.e. Cl	0.123	420	280.0	11.6	23	1173	18
D	40 m.e. Cl	0.180	619	504.0	7.3	15	706	10
Plant no. 2								
A	Trace Cl	0.016	215	67.3	9.3	29	2062	37
B	10 m.e. Cl	0.091	317	222.0	8.7	27	1790	32
C	20 m.e. Cl	0.123	420	306.0	7.2	22	1090	18
D	40 m.e. Cl	0.180	619	502.0	7.2	22	827	13

greater uptake of these nutrient ions as did the lower concentrations of chloride, calcium, magnesium, and sodium.

Experiment 3 (table III) included duplicate tests with corn grown in solutions containing, respectively, a trace, 10, 20, and 40 m.e. of chloride. The plants showed depressed root growth and lower water-uptake from the successively stronger solutions. A relatively greater effect on water uptake than on root growth resulted (fig. 2).

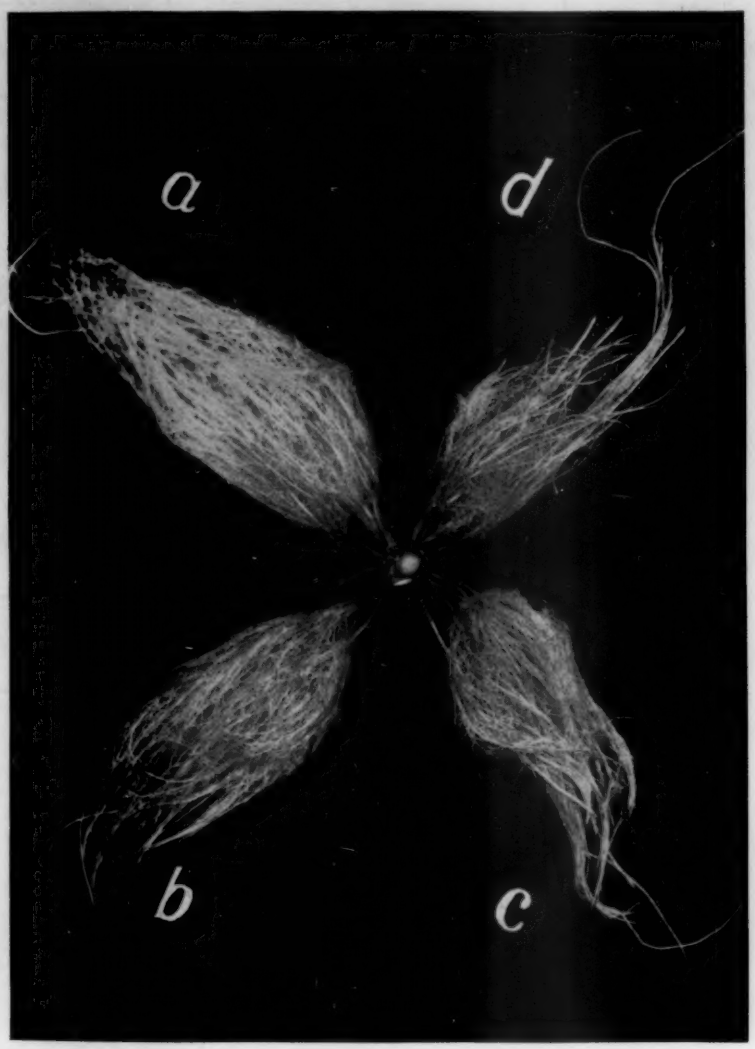

FIG. 2. Roots of a corn plant divided between solutions with: a, trace; b, 10 m.e.; c, 20 m.e.; and d, 40 m.e. chloride. Plant 1, Experiment 3.

Experiments 4 and 5 were conducted for the purpose of determining if sulphate would have an effect similar to chloride on root growth and water uptake. The chloride and sulphate concentrations were selected to give roughly similar differences in the osmotic pressures of the solutions compared. The resulting data (table IV) indicate no pronounced difference between the effects of chloride and sulphate salts at similar osmotic pressures.

TABLE IV

CORN PLANTS WITH ROOTS DIVIDED BETWEEN SOLUTIONS WITH ADDED CHLORIDE (EXPERIMENT 4), AND ADDED SULPHATE (EXPERIMENT 5)

CONTAINER	SOLUTION DESIGNATION	NEW SOLUTION		NEW ROOTS		WATER UPTAKE	
		△	$K \times 10^{-5}$ AT 25° C.				
		°C.		gm.	%	ml.	%
Experiment 4. Corn plant with roots in base nutrient with 2 chloride concentrations. Duration 13 days							
A	10 m.e. Cl	0.090	317	2.7	69	306	72
B	25 m.e. Cl	0.138	472	1.2	31	119	28
Experiment 5. Corn plant with roots in base nutrient with 2 sulphate concentrations. Duration 13 days							
A	20 m.e. SO_4	0.097	359	3.6	78	294	68
B	40 m.e. SO_4	0.150	561	1.0	22	139	32

TABLE V

CORN AND TOMATOES WITH ROOTS DIVIDED BETWEEN 3 × BASE NUTRIENT AND 0.5 × BASE NUTRIENT WITH ADDED CHLORIDE. EXPERIMENTS 6 AND 7

CONTAINER	SOLUTION DESIGNATION	NEW SOLUTIONS		NEW ROOTS		WATER UPTAKE	
		△	$K \times 10^{-5}$ AT 25° C.				
		°C.		gm.	%	ml.	%
Experiment 6. Corn plant with roots in concentrated base nutrient and dilute base nutrient plus chloride. Duration 13 days							
A	3 × base nutrient	0.157	583	1.8	51	146	54
B	0.5 × base nutrient plus 50 m.e. Cl	0.183	626	1.7	49	125	46
Experiment 7. Tomato plant with roots in concentrated base nutrient solution and dilute base nutrient solution plus chloride. Duration 17 days							
A	3 × base nutrient	0.167	599	4.1	45	435	53
B	0.5 × base nutrient plus 50 m.e. Cl	0.163	534	5.1	55	425	47

Experiments 6 and 7 (table V) were conducted to give further information on the question of whether the previously observed effects were the result of the action of specific ions or primarily reactions related to the total osmotic pressures of the solutions. In these experiments the behavior of corn and tomato plants was observed when their roots were divided between two solutions similar with respect to osmotic pressure but unlike in that one was concentrated base nutrient and the other dilute nutrient plus enough chloride to produce a similar total concentration. The effects of the two solutions were much alike with both corn and tomato. This finding may warrant the conclusion that osmotic relations are primarily involved but, if so, the fact should not be overlooked that nitrate was the principal anion (45 m.e.) in the 3 × base nutrient.

Separation of effects of root area and osmotic pressure on water uptake

At the beginning of each of the foregoing tests the number of roots in the several solutions of an experiment were nearly the same whereas by the end of the experiments there were more roots in the dilute than in the concentrated solutions. Experiments 8 and 9 (table VI) were undertaken to

TABLE VI

Differentiation of effects of new root development and osmotic pressures on water uptake. Experiments 8 and 9

Container	Solution designation	New solution		New roots		Water uptake	
		Δ	$K \times 10^{-6}$ at 25° C.				
		°C.		gm.	%	ml.	%
Experiment 8. Corn plant with roots in different concentrations of base nutrient solutions. Duration 13 days							
A	0.5 × base nutrient	0.025	113	3.6	62	451	76
B	3.0 × base nutrient	0.153	581	2.2	38	146	24
Experiment 9. Same as experiment 8 but positions of roots alternated between the two solutions every second day							
A	0.5 × base nutrient	0.025	113	.....	.....	319	65
B	3.0 × base nutrient	0.153	580	.....	.....	171	35

obtain an appraisal of the effects of solution concentration on water uptake that would be independent of number of roots. The first of these experiments (number 8) was conducted in the same manner as those that had pre-

ceded it; in experiment 9, however, the positions of the roots in the two solutions were reversed each second day; *i.e.*, the roots in container A were placed in container B and vice versa. By thus alternating the two halves of the root system between the concentrated and dilute solutions it was possible to maintain essentially equal root areas in the two solutions throughout the experimental period. At the end of experiment 9 one group of roots weighed 32 and the other 36 grams; this is probably as close an agreement as could be expected when account is taken of the difficulties attached to dividing the roots of a plant at the beginning of a test period into two equal groups.

A three-fold difference in water-uptake resulted in experiment 8 where the combined effects of differences in root area and in solution concentration were represented. In experiment 9, however, where essentially equal root areas were presented in each of the solutions, there was approximately a two-fold difference in water uptake. This latter result, which can be regarded as representing the effect of solution concentration alone, will be considered further in the discussion and in figure 5.

Roots divided between nutrient solutions and distilled water

In the three succeeding experiments, tables VII and VIII, the roots of corn and tomato plants were divided between distilled water and 2 × base nutrient. The situation thus created is obviously complex since it is well known that distilled water is injurious to plant roots. The question remained, nevertheless, as to whether the injury to roots would be of such a character as to lessen the rate of water uptake and also whether there would be enough movement of salts from the roots to the distilled water to permit some growth after several days had elapsed.

True (**18**) has traced the development of views on the causes of injurious effects of distilled water on root growth from the observations of early physiologists, who thought the absence of nutrients was responsible, through the period when the ill effects were attributed to metals derived from stills. From his own work with lupine seedlings (**18**), he concluded that a causal connection between loss of electrolytes by roots and their falling growth rate was almost certain. Injury was not prevented in his experiments when sugar or sodium chloride was added to the solutions to give osmotic pressures equal to that of Potomac River water used as a control. Calcium chloride prevented injury. True, referring to work of True and Bartlett, points out that Canada field pea seedlings made a fairly healthy growth in distilled water despite the loss of considerable quantities of electrolytes to the outer solution.

Hibbard (**10**) found that a slow process of adjustment or acclimatization to distilled water fitted *Lupinus alba* seedlings to better growth than

thrusting them directly into it. Finding that root growth was increased when the distilled water was changed 4 times daily, he concluded that the roots were injured because they secreted a substance toxic to themselves. SCARTH (17) found that boiling distilled water in silica vessels to expel carbon dioxide changed the pH from 5.5 to 7.1 and reduced the toxicity to Spirogyra. The tests were made in covered beakers but mention is not made that air or light was thereby excluded and the pH of the distilled water after the algae had been in it for some hours is not given.

The water used in the present experiments was from a gas-heated BARNSTEAD still and it was stored in tin-lined copper tanks. It was not redistilled and it is not known to what extent the carbon black on the roots adsorbed traces of copper or other metals.

As shown by table VII the roots of both corn and tomato made a better

TABLE VII

CORN AND TOMATOES WITH ROOTS DIVIDED BETWEEN DISTILLED WATER AND 2 × BASE NUTRIENT. EXPERIMENTS 10, 11, AND 12

CONTAINER	SOLUTION DESIGNATION	NEW SOLUTIONS		NEW ROOTS		TOTAL WATER UPTAKE	
		Δ	$K \times 10^{-6}$ AT 25° C.				
		°C.		gm.	%	ml.	%
Experiment 10. Two corn plants. Duration 34 days							
A	Distilled water	.....	.45	4.3	11	588	12
B	2 × base nutrient	0.113	413.00	34.0	89	4126	88
Experiment 11. Two tomato plants. Duration 17 days. Stems not split							
A	Distilled water	.....	.45	10.3	38	1175	42
B	2 × base nutrient	0.113	413.00	16.7	62	1605	58
Experiment 12. Two tomato plants. Duration 34 days. Stems split up about 5 cm.							
A	Distilled water	.....	.45	3.8	9	2886	30
B	2 × base nutrient	0.113	413.00	39.0	91	6647	70

growth in the nutrient solution than in the distilled water and for the full duration of the experiment more water was taken up from the solution of higher concentration. During the first days of all three experiments, however, relatively more water was removed from the distilled water vessels (table VIII) than from 2 × base nutrient; thus indicating that the injury to the roots—whatever its cause or character—did not prevent what was apparently a normal uptake of water. Later, when new and actively grow-

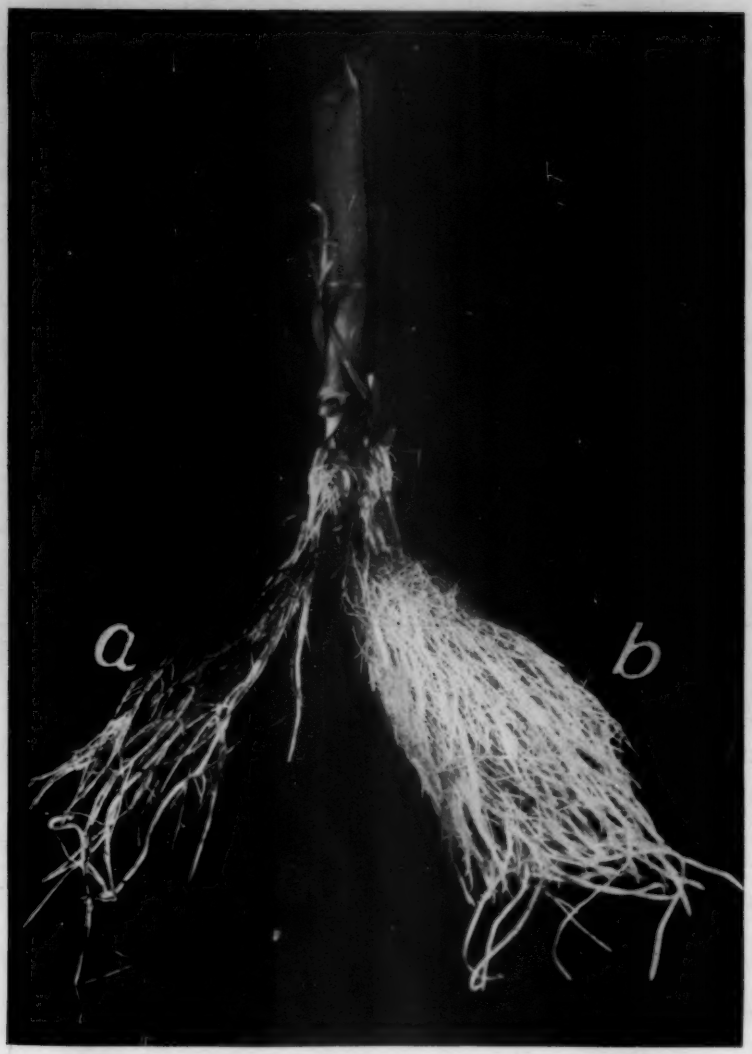

FIG. 3. Corn roots. a, in distilled water; b, in 2× base nutrient.

ing roots had developed in the containers with nutrient solution, the rate of uptake from the nutrient solution exceeded that from the distilled water. The original roots constituted a greater proportion of final roots in these tomato experiments than in the corn experiment and relatively more water was taken up from the distilled water by the tomatoes than by corn (figs. 3 and 4).

There is substantial evidence (table VIII) in each of these three experi-

TABLE VIII

WATER UPTAKE BY PERIODS AND CONDUCTANCE OF USED SOLUTIONS AT END OF PERIODS. EXPERIMENTS 10, 11, AND 12

CONTAINER	SOLUTION DESIGNATION	WATER USE BY PERIODS				CONDUCTANCES OF SOLUTIONS REMOVED		
		0 to 3*	0 to 10	11 to 17	18 to 34	10th	17th	34th
		ml.	*ml.*	*ml.*	*ml.*			
		Experiment 10. Corn						
A	Distilled water	90	145	79	364	9.22	0.99	1.32
B	2 × base nutrient	40	167	470	3489	356.00	305.00	296.00†
		Experiment 11. Tomato						
A	Distilled water	190	590	585	.....	6.64	0.96	.....
B	2 × base nutrient	140	750	855	.....	225.00	202.00	.....
		Experiment 12. Tomato						
A	Distilled water	190	405	595	1886	10.70	1.56	6.85
B	2 × base nutrient	90	345	918	5384	350.00	205.00	232.90†

* Approximate since solutions were not removed from containers for measurement.
† This solution renewed on 31st day—conductance not recorded.

ments indicating the movement of solutes from the roots into the distilled water; the nature of the electrolyte so moved, however, was not determined. A conductance of 10×10^{-5}, such as was observed in the first period of both experiments 10 and 12, corresponds to about 1 m.e. of cations per liter.

Discussion and conclusions

WATER RELATIONS

Experiment 9 provides a suitable basis for discussion of the effect of solution concentrations on water uptake by divided root systems. It was found that 1.86 ml. of water was withdrawn from the nutrient solution with an osmotic pressure of 0.3 atm. for each 1 ml. of water withdrawn from the nutrient solution with an osmotic pressure of 1.8 atm. Inasmuch as the characteristics of the two halves of the root system remained equal throughout the test it may be possible to estimate the magnitude of a maintained tension in the water in the base of the shoot that would be necessary to satisfy both of the two pairs of values (table VI, fig. 5).

The forces peculiar to living cells and tissue are neglected in the calculations that follow and it becomes of interest for this reason to consider to what extent such omissions are justified. Guttation is believed in some

FIG. 4. Tomato roots. a, in distilled water; b, in 2 × base nutrient.

instances to represent glandular action; MASON and PHILLIS (**13**) have shown that the osmotic pressures of protoplasmic saps are greatly in excess of vacuolar saps. This suggests that forces originating through respiratory activity of protoplasm may be important in the secretion of dilute solutions; even possibly of those passing from the root cortex into the xylem. Evidence from experiments by KRAMER (**12**) and others, however, support the view that forces arising from metabolic activity of root cells that tend to

move water into and through roots is negligible or quite secondary to forces created as a result of transpiration. It is the writer's opinion that exudation from decapitated plants (and exudation does not always occur) represents in large measure water movement brought about by the osmotic pressure of the tracheal sap when it is in excess of the sum of the osmotic pressure of the soil solution and capillary forces of the soil. In an intact plant even this force is of doubtful significance, except possibly during the recovery of turgidity, since the osmotic pressures in the foliar cells are greater than those of tracheal saps. The movement of water to leaves during transpiration (and after transpiration before turgidity has again been established) can be looked upon most logically as being the result of osmotic forces in excess of the inwardly directed forces exerted by cell walls. Plant roots can not be likened either to cylindrical tubes or to a porous medium such as soil. It is still probable, however, that the movement of water through roots obeys the law that has been found to hold in these other materials.

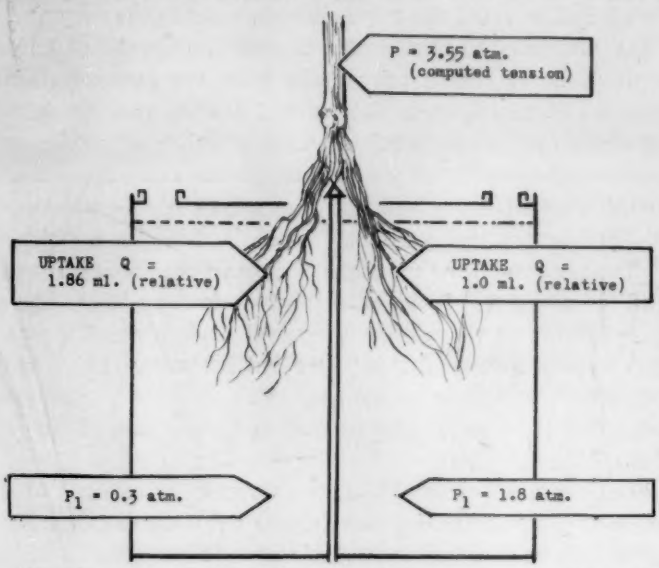

FIG. 5. Experiment 9. Water uptake, osmotic pressure, and tension relationships.

The flow of liquids through cylindrical tubes and through porous media has been found by POISEUILLE and DARCY, respectively, to be directly proportional to the difference in the hydraulic head of the liquid. For an approximate solution to the present problem, the most reasonable assumption we can make is that a similar relation holds for the transport of liquids through the conducting tissue of the plant. On the basis of this assumption and under present conditions where the gravity force is negligible compared with the pressure gradient force, one may write

$$Q = K (P - P_1)$$

where Q is the volume of flow in unit time, and $P - P_1$ is the difference in the pressure between the liquid in the conducting system and the liquid surrounding the roots. It is further assumed that osmotic and hydrostatic pressure differences are equally effective in producing the flow. The single value for the tension, P, of the water in the base of the shoot that satisfies both sets of water uptake and osmotic values is 3.55 atm. This value was obtained by substituting the two sets of experimental data in the above equation to obtain $1.86 = K(P - 0.3)$ and $1.0 = K(P - 1.8)$. Solving these equations simultaneously gives $P = 3.55$ atmospheres.

The foregoing value is necessarily an average one just as the water uptake values were the average of day and night uptake over the period of the experiment. With high transpiration rates the tension in the stem would become large, whereas at night as the plant regained full turgidity the tension would approach zero or become a positive hydrostatic pressure. From these relations it follows that as the tension increased the differences in the rate of uptake from the two solutions would narrow. On the foregoing basis, a tension of 10 atm. should produce an uptake of 1.15 ml. from the dilute solution for each 1 ml. uptake from the concentrated solution. As the tension fell to approach the value 1.8 atm., water would be withdrawn only from the dilute solution. Theoretically, at yet lower tensions there should be a movement of water through the roots from the dilute to the concentrated solution. It was not determined if this was the case, but BREAZEALE (**3**) has reported such flow through roots from moist soils into dry soils. The tension on the moisture of soils in the wilting range is about 16 atm., and according to recent experimental data by L. A. RICHARDS the soil moisture tension at the moisture equivalent is about 0.5 atmospheres. It is of some interest to note at this point that KEARNEY (**11**) found salts to have no effect on the wilting coefficient. This implies that if salts in the soil solution affect the tension between soil and plant there is some compensating effect within the plant.

It has been repeatedly found that the water requirements of plants on single solutions high in nutrient, chloride, or sulphate salts tend to be substantially lower than those of control plants. It has seemed more reasonable to attribute this to reduced transpiration rates resulting from salt effects within the leaves than to reductions in the osmotic differentials between the leaves and substrate.

ROOT DEVELOPMENT

The greater development of roots in the dilute than in the concentrated solutions (distilled water experiments as exceptions) might be attributed: (1), to conditions unfavorable to root growth in the concentrated solutions; (2), to conditions particularly favorable to root growth in the dilute solutions; or (3), to factors within the roots that caused more of the organic

solutes translocated downward through the stem either to move into the dilute-solution roots or, having moved in, to be elaborated into tissue more rapidly. Either of these latter explanations would imply that the total growth of the root system as a whole had not been affected.

The fact that chloride, sulphate, and concentrated nutrient salts had similar effects would tend to discount the view that the concentrated solutions were especially unfavorable for root growth if all of the roots had been in one or the other kind of solution. It was noted in connection with experiment 1, where there was only a negligible root growth in the solution with 50 m.e. of chloride, that in other experiments with corn the roots of plants with all roots in 50 m.e. of chloride constituted a greater proportion of the weight of entire plants than was the case with plants growing with all roots in control solutions. In experiment 3 no appreciable difference was found in the moisture content of roots in the dilute and concentrated solutions.

In the absence of measurements on the distribution and concentrations of carbohydrates and salts in the two sets of roots of any experiment, discussion cannot be extended to the pertinent question of whether salt accumulations in the root tissues in the concentrated solutions tended to deflect the movement of organic materials from the shoots into the roots growing in the dilute solutions. A reason for a more rapid elaboration of synthesized products into cell structure in the roots containing less salt is not evident but the possibility that such was the case remains. A more rapid utilization of these materials by the dilute-solution roots would tend to establish more favorable translocation gradients to them.

As a final consideration some attention may be given to relations between the turgor pressures of roots and root growth. Cell growth by enlargement must be related to turgor pressure but one might question the extent to which the laying down of wall materials would be similarly influenced. ROBERTS (**16**) found the concentration of the cell sap of root hairs of radish growth in a series of 0.02 M to 0.65 M sucrose solutions to increase with the concentration of the external medium and maintain osmotic pressures 4 to 6 atm. above it. As further evidence of the adjustment of the plant to its substrate EATON (**6**) has presented the results of cryoscopic measurements on the expressed sap of a series of crop plants grown on saline solutions. The expressed sap of leaves of tomatoes, from the same seed stock as the present plants, when grown in out-of-doors sand cultures maintained osmotic differentials of 9.4, 8.9, 8.8, 9.5, 9.3, and 9.3 atm., respectively, above nutrient solutions as follows: control, plus 50 and 150 m.e. chloride, and plus 50, 150, and 250 m.e. sulphate. With this indication that the osmotic differentials between the root cells or leaves of a plant and its substrate are not greatly changed, within reasonable limits, by solutes added to the substrate, any conclusion with respect to disadvantages in water relations of the saline roots would have to be arrived at on some other basis. Otherwise,

one might reason that since accumulation ratios are relatively high when plants are on dilute media and low when on concentrated media, that higher turgor pressures would exist in the dilute-solution roots than in the concentrated-solution roots. In other words, whatever the cause of the lesser root growth in the concentrated solutions may be, it is not reasonable to assign the cause to lack of turgor in the cells of the concentrated-solution roots.

Agronomic aspects

Prominent among the factors that tend to create differences in the concentration of the soil solution from point to point in irrigated soils there are especially to be mentioned: (1), variations in the permeability of proximately situated soil zones, even though of similar texture; and (2), the usually greater uptake by the plant of water than of salt, thereby increasing the concentration of the soil solution. The first-mentioned consideration gives rise to much lateral diversity since the less permeable zones are not leached as effectively as the permeable zones. The second consideration is largely responsible for the pronounced tendency for salt concentrations to increase with depth. The portion of a soil solution that is residual from a particular irrigation tends to be displaced downward in successive steps by successive irrigations and rains; thus the unused fraction ultimately lies deep in the root zone with a greatly reduced volume but still containing much of its initial salt.

Among other factors that influence both the average concentration of the soil solution as well as variations within the root zone, there are to be mentioned such considerations as the quality of the irrigation water, the extent to which water is used in excess of crop needs to promote leaching of the salts from the root zone, the amount of rainfall, methods of applying water to the land, and subsoil drainage conditions. Salt impregnated layers of clay as well as saline water tables are sometimes the source of salt brought into the upper root zone with rising moisture.

Both lateral and vertical diversity were illustrated in table I. As features pertinent to that example, it is to be noted that it had been the owner's policy to apply only sufficient irrigation water to maintain the trees from one irrigation to the next. Since the rainfall over a period of years prior to the collection of those samples had been subnormal there had been little recent leaching of the lower root zone. After two winters with leaching rains a much more uniform salt distribution was found. The irrigation water was of good quality containing but 0.29 m.e. of sulphate and 0.48 m.e. of chloride per liter. The soil samples were collected from beneath the irrigation furrows nearest the trees.

Summary

Corn and tomato plants were grown with their roots divided between two or more solutions of unequal concentration. They developed more

roots in the dilute than in the concentrated solutions and the water uptake from the former exceeded that from the latter. The foregoing was found irrespective of whether the differences in concentration were affected by the addition to the base nutrient of chloride, sulphate, or additional nutrient salts. Little difference in water uptake or in root growth resulted in solutions of similar osmotic pressure when one was high in chloride and the other in complete nutrient salts. These findings indicate that osmotic pressures, rather than specific ion effects, are primarily involved.

With an equal growth of the two halves a divided root system accomplished by alternating their positions each second day between dilute and concentrated nutrient solutions (0.3 and 1.8 atm.), 1.86 ml. of water was taken up from the dilute solution for each 1 ml. of water from the concentrated solution. A value of 3.55 atm. for the tension in the water of the stalk was found by calculation to satisfy these two sets of values on the basis of a first assumption that the observed water uptake by the two halves of this root system should be proportional to the difference in the osmotic pressure in the solutions and the tension in the water in the stem. This value is of course an average one and the data are discussed accordingly.

In an experiment parallel with the foregoing one, the two portions of the root system remained throughout in the solutions in which they were started. The new roots developed in the dilute solution weighed 1.6 times as much as those in concentrated solution and 76 per cent. of the total water taken up by the plant was from the dilute solution.

Roots divided between distilled water and concentrated nutrient made more growth in the latter. Almost twice as much water was withdrawn from the distilled water vessels during the first three days of the experiment as from the nutrient solution but this was later reversed.

The investigations were undertaken as a means of appraising the effects of the variability in the concentration of the soil solution often found within the root zone of plants growing on irrigated lands. When soils are permeable and extensively leached this variability may be slight but under other circumstances it may be great. An example is presented of the situation found in a permeable but lightly irrigated orchard soil wherein 15- to 60-fold variations in the concentrations of sulphate and chloride, respectively, were found within the root zone of single trees.

The writer acknowledges with appreciation the assistance of G. Y. BLAIR in this investigation.

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LITERATURE CITED

1. AUCHTER, E. C. Is there normally a cross transfer of foods, water and

mineral nutrients in woody plants? Maryland Agr. Exp. Sta. Bull. 257. 1923.

2. ————————, and SCHRADER, A. L. Influence of shade on the behavior of apple trees. Proc. Int. Congress Plant Sci. **2**: 1056–1069. 1926.
3. BREAZEALE, J. F. Maintenance of moisture-equilibrium and nutrition of plants at and below the wilting percentage. Arizona Agr. Exp. Sta. Tech. Bull. 29. 137–177. 1930.
4. CALDWELL, J. Studies in translocation. Movement of food materials in the Swede turnip. Proc. Roy. Soc. Edinburgh **50**: 130–141. 1930.
5. ————————. Studies in translocation. II. The movement of food materials in plants. New Phytol. **29**: 27–43. 1930.
6. EATON, F. M. The toxicity and accumulation of chloride and sulfate salts in crop plants. Jour. Agr. Res. (In press). 1941.
7. FURR, J. R. The cross-transfer of water in mature lemon trees. Proc. Amer. Soc. Hort. Sci. **30**: 45–51. 1933.
8. GILE, P. L., and CARRERO, J. O. Absorption of nutrients as affected by the number of roots supplied with the nutrient. Jour. Agr. Res. **9**: 73–95. 1917.
9. ————————, and ————————. Assimilation of nitrogen, phosphorus, and potassium by corn when nutrient salts are confined to different roots. Jour. Agr. Res. **21**: 545–573. 1921.
10. HIBBARD, R. P. The question of the toxicity of distilled water. Amer. Jour. Bot. **2**: 389–401. 1915.
11. KEARNEY, T. H. The wilting coefficient for plants in alkali soils. Bur. Plant Ind. Circular 109. 17–25. 1918.
12. KRAMER, P. J. The forces concerned in the intake of water by transpiring plants. Amer. Jour. Bot. **29**: 784–791. 1939.
13. MASON, T. G., and PHILLIS, E. Experiments on the extraction of sap from the vacuole of the leaf of the cotton plant and their bearing on the osmotic theory of water absorption by the cell. Ann. Bot. n.s. **3**: 531–544. 1939.
14. MCMURTREY, J. E. Cross transfer of mineral nutrients in the tobacco plant. Jour. Agr. Res. **55**: 475–482. 1937.
15. MILLER, E. C. Plant physiology. McGraw-Hill Book Co., New York. 1938.
16. ROBERTS, EDITH A. The epidermal cells of roots. Bot. Gaz. **62**: 488–506. 1916.
17. SCARTH, G. W. The toxicity of distilled water and its antagonism of cations. Trans. Roy. Soc. Canada Sec. V **18**: 97–101. 1924.
18. TRUE, RODNEY H. The harmful action of distilled water. Amer. Jour. Bot. **1**: 255–273. 1914.

INFLUENCE OF SOIL MOISTURE ON PHOTOSYNTHESIS, RESPIRATION, AND TRANSPIRATION OF APPLE LEAVES[1]

G. William Schneider and N. F. Childers

(WITH THREE FIGURES)

Introduction

A deficiency of water under natural and even cultural conditions is probably responsible for the poor growth and death of more plants than diseases, insects, or any other cause. Although it is possible to see with the naked eye how and when a plant responds to low water supply, it is of interest to know the extent to which a drying soil affects metabolic processes before and during the time wilting is visible. It is of interest also to know whether a wilted plant recovers immediately in leaf activity when water has been supplied to the soil, or if normal activity is delayed or never again attained. The results presented in this paper trace the daily effect of a gradually drying soil on apparent photosynthesis, respiration, and transpiration of small apple trees as the soil in which they were growing gradually dried to approximately the wilting percentage, after which it was watered to field capacity.

Methods

Environment-control chamber experiments

In the Horticulture and Forestry Building, Ohio State University, a large insulated chamber (**6**) seven by eight by eight feet high, inside dimensions, has been constructed for the purpose of controlling light, temperature, and relative humidity while leaf metabolism tests are being made. At the time of these experiments no effort was made to control the amount of carbon dioxide in the air. The air replacing that removed by the pump was drawn from the outside of the building. Carbon dioxide in the air usually averaged about 15 to 17 milligrams per cubic foot, corrected to standard temperature and pressure.

The apparatus used and the procedure followed to determine the carbon dioxide exchange was similar to that previously described by Heinicke (**9**) and Heinicke and Hoffman (**11**) except that a mercury piston air pump[2] was used to draw the air over the leaves. This method measures the amount of carbon dioxide absorbed by the leaf and is therefore a measure of *apparent* photosynthesis and not *actual* photosynthesis. Thus whenever the term

[1] This paper has been published in abstract elsewhere (**7**).

[2] Thanks are expressed to Dr. A. J. Heinicke, Cornell University, who devised the pump and granted us special permission to use it in our experiments.

photosynthesis is used in this paper it should be considered as meaning apparent photosynthesis.

The Stayman and McIntosh apple trees used in the chamber experiments were two years old and growing in five- and ten-gallon glazed stone crocks. The soil in these containers had a moisture equivalent of 36.4 per cent. and a wilting percentage of 15. 2 (determined by analyzing the soil after dwarf sunflowers had grown in it to about 10 inches in height, then allowed to wilt). The trees had been cut back to 8-inch stubs when set in the greenhouse in December, 1938, and one vigorous shoot was allowed to grow from each stub. Six leaves were selected for study on the check tree and six on the test tree, all of which were evenly spaced around the three- to four-foot shoots. Terminal buds did not form on any of the test trees during periods of experimentation.

Daily determinations started at 11:00 A.M. and were run for three hours, immediately after which several respiration determinations were made at times specified later. To make a respiration determination the lights were turned off and an office heating cone, which had been attached to an oscillating fan, was turned on to help maintain with the cooling system a constant temperature level.

The entire bank of lights was usually turned on about 7:45 A.M. each morning. They were turned off between 5:00 and 6:00 P.M. since earlier tests have indicated that small apple trees should receive equal amounts of light each day if a fairly consistent relationship in leaf activity from one day to the next is maintained between two trees.

Transpiration was measured by a method similar to one described previously (**10**). The system consisted of several Freshman bottles (2 inches in diameter by 4½ inches high, one for each leaf and air-check line) which contained ground pumice stone impregnated with sulphuric acid. The acid efficiently removed water vapor from air flowing through 9-mm. glass tubing at the rate of three to four cubic feet per hour, and did not interfere with the carbon dioxide to be removed later in the absorption towers. After each determination the rubber stoppers, with glass attachments and metal numbers, were removed from the bottles which, in turn, were placed in a drying oven at about 160° C. After 24 hours the bottles were removed and allowed to cool to air temperature, stoppered, and held ready for the next run. These bottles could be used for a month or more until the absorption medium became somewhat weak and it was necessary to impregnate the stones again with sulphuric acid. The common laboratory procedure for preparing these stones was essentially as follows: Place pumice stones about the size of peas in an evaporating dish or casserole and cover with concentrated sulphuric acid. Place on a hot plate and heat until dense white fumes appear, then continue the heating for one-half hour. Allow to cool and drain off all of

the acid. Again heat the pumice until heavy white fumes of sulphur trioxide are given off. At the end of this heating, there should be no excess of sulphuric acid present. Cool in a desiccator and store in stoppered glass containers.

General procedure

The general procedure employed for the water deficiency experiments was essentially as follows: Determinations of apparent photosynthesis, transpiration and, in some cases, respiration were made from three to five days before the dry period began in order to establish a relationship in leaf activity between the group of leaves to be used as test leaves and those to remain as controls. When a consistent relationship had been established, the test tree ceased to receive water while the check, or control, tree received it daily in order to keep the soil at approximately the field capacity. Daily determinations were continued until the test plant had reached the incipient or permanent wilting stage (as specified later) when the soil was again watered to field capacity and the runs continued for 7 to 10 days. Stomatal observations were made either by removing small sections one centimeter square from neighboring leaves on the shoot and immediately inspecting them under the microscope, or by observing the inverted intact leaf through a mounted microscope.

Field experiments

The assembly of carbon dioxide absorption towers, electric air pump (instead of mercury piston pump), and wet test meter were located in the greenhouse and connected by glass tubing to individual leaves on two Stayman apple trees. The trees were growing in five-gallon glazed stone crocks outside the greenhouse where they were fully exposed to the sun. Soil surrounding their root systems had a moisture equivalent of 38.4 per cent. and a wilting percentage of 15.55. Leaf cups were attached to six leaves on the test plant and to four leaves on the check while two of the air lines were reserved to determine the amount of carbon dioxide in air before it reached the leaves. Leaves selected on each tree were evenly distributed up and down and around the four- to five-foot shoots. Two four-hour determinations were usually made each day, the first beginning about 7:00 A.M. and the second about 11:30 A.M. Light and temperature readings were recorded at the beginning, middle, and end of each run with a Weston illuminometer and a Fahrenheit dairy thermometer.

Results

Environment-control chamber

Four experiments were performed in the environment-control chamber. In the first test only apparent photosynthesis determinations were made; in

the second and third experiments apparent photosynthesis, respiration, and transpiration data were recorded; in the fourth only respiration was studied.

EXPERIMENT I (APPARENT PHOTOSYNTHESIS).—The trees used in this experiment were of the McIntosh variety; tree A was the check and tree B the test plant. During each determination the temperature was held at 80° F., and light intensity was constant at an average of about 4000 foot candles for all leaves concerned. Relative humidity was approximately 30 per cent. during the light period. A relationship between the photosynthetic activity of trees A and B was established in the eight-day period from February 14 to February 21. The data in table I show the daily rate of apparent photosynthesis in milligrams of carbon dioxide absorbed per 100 square centimeters of leaf surface per hour, before and after the dry period. The test tree, B, received its last watering the night of February 20, while the check tree, A, received it daily throughout the experiment. The leaf relationship during the standardization period showed that for every 100 milligrams of carbon dioxide assimilated by the check plant the test plant utilized 128.9 milligrams. Thus, the *expected rate* of the test plant was 128.9 per cent. of the check (see foot-note in table I for explanation of figures termed *expected rate and percentage expected rate*). During the first four days of the dry period from February 22 to 25, the test plant showed an average increase in carbon dioxide assimilation of about three per cent. above its rate previous to the treatment. From February 25 until March 3 there was a gradual decrease in the rate of photosynthesis of tree B from 101 to 76 per cent. of the expected rate. At no time during this period did the test tree show signs of wilting, even by late afternoon when the lights were usually turned off. On March 4 there was a sharp reduction in photosynthesis, to 46 per cent. of the expected rate; no wilting was evident, however, until about three hours following the determination when the leaves appeared to be slightly flaccid. The next day, March 5, the test tree was fairly turgid at the start of the run but was wilted at the end of the determination, with the top leaves showing the greatest effect of water deficiency. The entire tree showed signs of wilting on the following morning of March 6 when the lights were turned on. The soil continued drying until March 11 when assimilation had gradually fallen to around 14 per cent. of the expected rate. Stomata on the test tree appeared to be closed continuously during the period of wilting, including the mornings before determinations were started. It is interesting to note that fairly good values for photosynthesis were obtained when the leaves were somewhat wilted and the stomata appeared to be closed. On March 11, about two hours before the determination, water was added to the dry plant and by starting time the tree had almost recovered to complete turgidity. Within another hour the stomata were almost as far open as were those on the check leaves (75 to 100 per cent.).

TABLE I

EFFECT OF SOIL MOISTURE ON THE RATE OF PHOTOSYNTHESIS OF McINTOSH APPLE LEAVES. EXPERIMENT I, TEMPERATURE 80° F.

DATE, 1939	APPARENT VALUES OF PHOTOSYNTHESIS IN CO_2/100 CM^2/HR.		EXPECTED RATE OF TEST TREE
	CONTROL	TEST	
	mg.	*mg.*	%
Feb. 14	31.4	37.7	.....
15	26.8	29.6	.....
16	21.7	30.3	.....
17	23.0	33.0	.....
18	12.9	15.2	.....
19	17.6	26.1	.....
20*	15.4	24.4	.....
21	19.7	21.6	.....
Average	21.1	27.2	128.9‡
22	11.2	15.0	103.9§
23	15.2	20.1	102.6
24	11.8	16.0	105.1
25	12.5	16.3	101.2
26	18.9	19.1	78.4
27	14.0	11.1	61.4
Mar. 1	7.6	7.5	76.5
2	12.4	12.2	76.3
3	15.7	15.4	76.1
4	8.7	5.0**	44.6
5	20.8	15.4	49.7
6	13.6	5.4	30.8
7	9.1	5.4	46.0
8	15.9	2.3	11.2
9	9.2	2.2	18.5
10	12.4	3.2	13.8
11†	10.3	8.8	66.3
12	10.3	8.5	64.0
13	12.2	16.5	104.9
16	10.0	14.9	115.6
17	10.5	13.7	101.2
18	16.0	20.4	98.9
19	11.1	14.9	104.1
20	8.6	10.9	98.3
21	10.5	15.5	114.5
22	15.9	17.9	87.4
23	7.9	8.6	84.5
24	11.9	13.8	90.0
25	12.6	14.2	87.4

* The test plant last received water on the evening of February 20.

† Test plant watered to field capacity of the soil two hours before determination was made.

‡ Obtained by dividing the average apparent photosynthetic value (27.2) for the test tree by the average value (21.1) for the check tree and multiplying by 100.

§ Obtained by dividing the daily rate of photosynthesis of the test tree (15.0) by the daily rate of photosynthesis of the check plant (11.2), multiplying by 100 and dividing the product by 128.9.

** Test tree showed first signs wilting two hours after run.

Assimilation during this run recovered to 66 per cent. of its expected rate and within 48 hours had returned to its original status.

From March 11 to 15 the test tree was watered daily and then allowed to dry again. During the six-day period which followed March 15, apparent photosynthesis of the test plant averaged 105.4 per cent. of its expected rate; or, in other words, it showed a 5 per cent. increase which corresponded with the average 3 per cent. increase obtained at the beginning of the first dry period from February 22 to 25. Beginning with March 22, however, photosynthetic activity began to decrease at a rate similar to the first test. The experiment was discontinued.

EXPERIMENT II (APPARENT PHOTOSYNTHESIS, RESPIRATION, AND TRANSPIRATION).—Stayman Winesap trees were used in this experiment. Temperature in the chamber during each determination was constant at 90° F. and

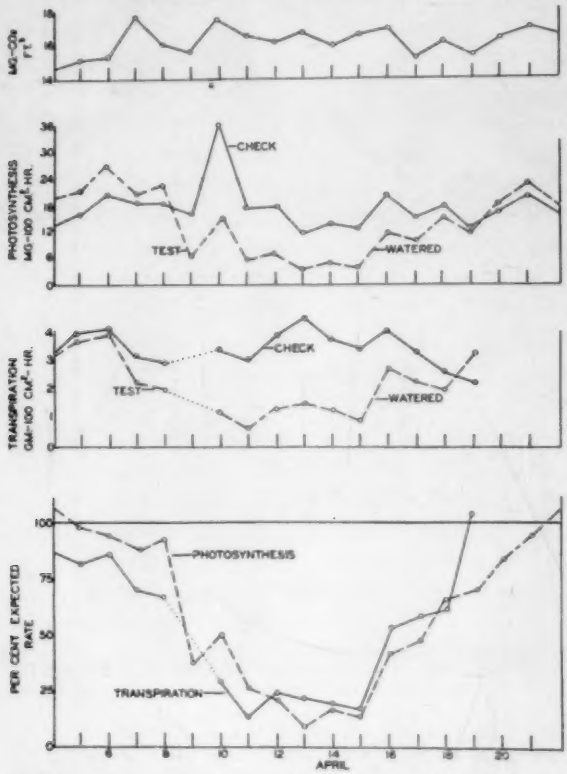

FIG. 1. The influence of a gradually drying soil on apparent photosynthesis and transpiration of apple leaves. The test tree was last given water the evening of April 1. First wilting occurred the afternoon of April 10; soil was watered to field capacity two hours before the determination on April 16 (experiment II).

light averaged approximately 4000 foot candles for the six test and six check leaves. These factors did not vary during the nine-hour day from 8:00 A.M. to 5:00 P.M. At other hours of the day the lights were turned off and the plants remained at room temperature which was about 75° F.

Data for this experiment are presented in table II and graphically shown

TABLE II

INFLUENCE OF SOIL MOISTURE ON PHOTOSYNTHESIS, RESPIRATION, AND TRANSPIRATION OF STAYMAN APPLE LEAVES. EXPERIMENT II, TEMPERATURE 90° F.

Date, 1939	Apparent values per hour per 100 cm^2						Expected rate of test tree*	
	Assimilation CO_2		Transpiration H_2O		Respiration CO_2			
	Control	Test	Control	Test	Control	Test	Assim.	Transp.
	mg.	*mg.*	*gm.*	*gm.*	*mg.*	*mg.*	%	%
Mar. 27	22.1	26.7	1.9	2.0	.....	.....	.....	.....
28	14.1	20.1	.....	.....	.....	.....	.....	.....
29	12.9	17.1	3.2	3.4	.....	.....	.....	.....
30	22.6	30.7	2.9	3.7	.....	.....	.....	.....
31	19.4	25.9	2.7	3.6	.....	.....	.....	.....
Apr. 1†	17.8	22.8	3.5	3.7	.....	.....	.....	.....
2	16.2	22.1	3.3	3.9	.....	.....	.....	.....
Average	17.9	23.6	2.9	3.4	.....	.....	131.8	117.2
4	13.0	18.2	3.4	3.4	3.6	2.0	106.2	85.3
5	15.7	19.8	3.8	3.6	.....	.....	95.6	80.8
6	20.5	24.8	3.9	3.8	7.0	6.0	91.7	83.1
7	18.4	21.3	3.2	2.4	.....	.....	87.8	64.0
8	18.5	22.0	2.8	2.0	.....	.....	90.2	60.9
9	17.1	5.9	.....	.....	.....	.....	26.2	.....
10	36.4	17.6	3.3	1.0	.....	.....	36.6	25.9
11	18.6	4.2	2.9	0.5	2.6	4.6	17.1	14.7
12	18.6	5.4	3.5	1.0	5.5	6.5	22.0	24.4
13	11.9	1.4	4.2	1.0	.....	.....	8.9	20.3
14	13.8	2.5	3.3	0.6	1.5	3.5	13.7	15.5
15	12.6	2.2	3.2	0.5	4.7	5.2	13.2	13.3
16‡	19.9	11.7	3.8	2.3	3.3	4.0	44.5	51.6
17	14.7	9.5	3.1	1.8	3.8	4.8	49.0	58.0
18	16.5	14.7	2.5	1.6	4.2	5.0	67.6	54.6
19	13.0	12.7	2.4	2.9	2.9	2.9	74.1	103.1
20	14.6	16.6	.....	.....	3.6	3.4	86.3	.....
21	16.7	20.9	.....	.....	2.9	1.5	94.9	.....
22	10.5	14.7	.....	.....	.....	.....	106.2	.....

* See table I for method of calculation.

† Test tree last watered the evening of April 1. (No determination made first day of dry period, April 3.)

‡ Test tree thoroughly watered two hours before test period began.

in figure 1. The relationship between the control tree, C, and the test tree, D, was established in the seven-day period from March 27 until April 2. During this standardization period the test plant was found to have an expected photosynthetic rate of 131.8 per cent. and an expected rate of transpiration of 117.2 per cent. No determinations were made on April 3.

On April 4 the test tree showed an increase of 6 per cent. in photosynthesis. This increased rate of photosynthesis during the early part of the dry period apparently lasted at the most only two days. This shorter period of increased photosynthetic activity, as compared with the four-day period in the previous experiment, was probably due to the 10 degree higher temperature which caused a more rapid drying of the soil and a higher rate of transpiration by the leaves. It is interesting to note that while photosynthesis showed a six per cent. increase on April 4, transpiration showed a 15 per cent. decrease. On the following day, April 5, the percentage expected rate of apparent photosynthesis was about 4 per cent. below its rate previous to the test period; transpiration, however, showed about a 19 per cent. decrease. From April 5 until April 8 photosynthesis of the test tree showed a 19 per cent. decrease and transpiration a 40 per cent. decrease. It is apparent from these data that transpiration showed a marked drop two days before an appreciable reduction in photosynthesis occurred. The test tree showed slight wilting at the tip at the conclusion of the run on April 10. It had recovered, however, within two hours after the lights were turned off in the afternoon. In spite of the slight wilting which occurred at the end of this three-hour run the test plant assimilated carbon dioxide at the fairly good rate of 17.6 milligrams per 100 square centimeters of leaf surface per hour. On April 11 the lights were turned on about three hours before the run began, and at starting time the plant again showed signs of slight wilting at the tip. By termination of this run all leaves were drooping. Carbon dioxide assimilation was 17 per cent. of the expected rate while transpiration was only 15 per cent. of its expected rate. In the period from April 11 to 16 as the plant continued to dry, the photosynthetic activity and transpiration of tree C dropped in each case to about 13 per cent. when the soil in which it was growing had reached a definite wilting percentage.

About two hours before the run was started on April 16 the test tree was thoroughly watered but it did not recover to full turgidity until about three hours after the lights had been turned off in late afternoon. Although the plant was obviously wilted during this run, the rate of photosynthesis and transpiration increased to 44 and 52 per cent., respectively, of their original rate. By April 19 carbon dioxide assimilation of the test plant had increased to about 74 per cent. of its former rate, while transpiration showed a rapid increase to 106 per cent. On the day following this increase in transpiration, photosynthetic activity increased to 86 per cent., and by April 22 showed complete recovery to 106 per cent. of the expected rate. Thus, the time required for this plant to return to about normal transpiration and assimilation after the dry period was four and seven days, respectively.

A three-hour respiration determination was made on several occasions following the above daily tests. Conditions were maintained the same with

the exception that the chamber was darkened. Unfortunately no respiration runs were made before the start of the dry period. On April 4 and 6, however, respiration tests were made on the two sets of leaves when photosynthesis and transpiration had not as yet shown marked reductions. In table II it is apparent that the respiration rate of tree C, the test plant, was lower than that of the check tree, D, for both determinations. By April 11 when the next determination was made, however, respiration of the drying plant had increased above the check and this relationship continued during the dry period, and for four or five days after the water had been added on April 16. It is evident in table II that variations in the rate of respiration were directly opposite to variations in photosynthesis. When the former process showed an increase, the latter showed a decrease as the soil gradually dried; after water was added, the situation was slowly reversed.

EXPERIMENT III (APPARENT PHOTOSYNTHESIS, RESPIRATION, AND TRANSPIRATION).—This experiment was carried out under the same procedure and set of conditions as the previous experiment except the temperature was 10° F. higher (100° F.) and the test plant was allowed only to show incipient wilting before water was supplied. Tree E, the control, and tree F, the test, were both of the McIntosh variety. During the standardization period, April 24 to April 28, both trees were watered daily in the evening until the evening of April 28 when only the control tree received water and the test plant was allowed to begin drying. In this experiment, as in the previous two experiments, shortly after the last watering the test plant showed an increase in photosynthetic activity over a two-day period, April 29 and 30, or an average increase of about seven per cent. Transpiration also showed a slight increase on but one day, April 29. According to the data in table III, it is evident again that transpiration shows a decrease before photosynthesis when water becomes limiting. Transpiration dropped slightly on May 30, and photosynthesis showed a slight decrease the next day; the first marked drop in transpiration came on May 3 while the first marked drop in photosynthesis occurred the following day. Toward the end of the determination on May 6 the test plant showed signs of wilting but recovered about two hours after the lights were turned off in the evening. Photosynthesis and transpiration on this day were 44 and 36 per cent., respectively, of their expected rate. Soil in which the test plant was growing was watered to field capacity late in the evening of May 6. The next day photosynthesis had recovered to almost its original status and had completely recovered by May 8. Transpiration showed complete recovery by May 9.

Immediately following each of the above determinations the chamber was darkened and respiration runs were made at the same temperature (100° F.) on four leaves on each of the above test and check plants (see

TABLE III

THE EFFECT OF SOIL MOISTURE ON PHOTOSYNTHESIS, TRANSPIRATION, AND RESPIRATION OF APPLE LEAVES. EXPERIMENT III, TEMPERATURE 100° F.

DATE, 1939	APPARENT VALUES PER HOUR PER 100 CM^2						EXPECTED RATE OF THE TEST TREES*		
	ASSIMILATION CO_2		TRANSPIRATION H_2O		RESPIRATION CO_2				
	CONTROL	TEST	CONTROL	TEST	CONTROL	TEST	ASSIMILATION	TRANSPIRATION	RESPIRATION
	mg.	*mg.*	*gm.*	*gm.*	*mg.*	*mg.*	%	%	%
Apr. 24	14.6	12.2	2.5	2.7	.....	.....	.....	.....	.....
25	16.9	15.0	2.9	3.2	.....	.....	.....	.....	.....
26	20.6	19.5	3.0	3.2	6.3	4.1	.....	.....	.....
27†	20.6	19.7	3.3	3.6	4.6	3.6	.....	.....	.....
28	17.3	15.5	3.2	3.6	4.5	3.4	.....	.....	.....
Average	18.0	16.4	3.0	3.3	5.1	3.7	91.1	110.0	72.5
29	21.5	20.5	2.9	3.3	.....	.....	104.7	103.4	.....
30	16.2	16.0	3.5	3.8	.....	.....	108.4	98.7	.....
May 1	12.2	10.6	3.4	3.3	6.0	5.9	95.4	88.2	135.6
2	18.2	14.9	4.3	3.9	6.9	6.9	89.9	82.4	137.9
3	19.0	16.5	4.1	2.3	2.8	3.0	95.3	51.0	147.8
4	20.7	9.5	4.2	2.2	2.9	3.2	50.4	47.6	152.2
5	19.7	8.1	4.9	2.0	1.7	2.0	45.1	37.1	162.2
6‡	20.7	8.2	4.6	1.8	2.6	3.0	43.5	35.6	159.1
7	16.2	13.6	3.8	3.8	3.0	2.4	92.2	90.9	110.3
8	15.3	14.7	3.4	3.6	.....	.....	105.5	96.3	.....
9	12.5	11.8	3.2	3.7	4.0	2.8	103.6	105.0	96.6
10	18.8	17.4	.....	.....	.....	.....	101.6	.....	.....

* See table I for method of calculation.
† Test tree last watered evening of April 27.
‡ Test tree watered thoroughly evening of May 6.

experiment IV regarding the remaining four leaf cups which were attached to another drying plant for respiration studies). The data are given in table III and shown graphically in figure 2. It is apparent again that as

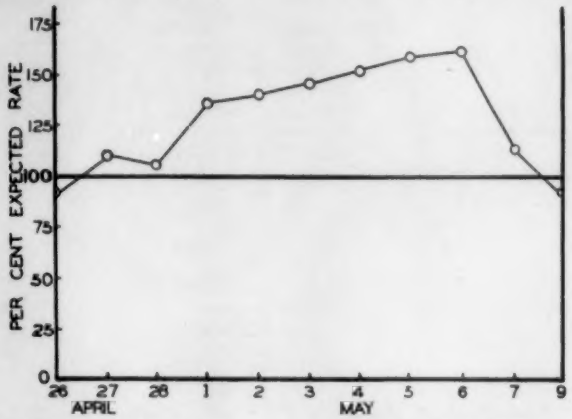

FIG. 2. The influence of soil moisture on respiration of apple leaves. The test tree received the last watering on the evening of April 27. It was watered again the evening of May 6 (experiment III).

water becomes limiting to an apple tree the respiration rate correspondingly increases, but when the plant has been watered again it gradually returns to its original relationship with the check plant. The highest rate of respiration, a 62 per cent. increase, was recorded on May 6 when transpiration and photosynthesis were lowest and the plant showed definite signs of wilting.

EXPERIMENT IV (RESPIRATION).—Data presented in table IV were collected on a McIntosh test tree, G, at the same time respiration determinations were made on tree F in experiment III. Leaf cups were attached to four leaves on test tree, G, while the control tree, E, remained as a reference in both experiments III and IV. The standard relationship between assimilation of the test and control leaves was established between May 4 and 6, and from the evening of May 6 to 9 inclusive the test tree received no additional water. In table IV it is evident that on the first day of the dry period, May 7, tree G showed the beginning of a gradual increase in respiration. On May 8, 9, and 10 there were further increases in respiration to 23, 34, and 30 per cent., respectively. After the test plant had been watered thoroughly the evening of May 10, it returned on May 11 to almost its original relationship in respiration with the reference tree. Thus, it is apparent from these data that when the dry period for an apple tree is short and less severe, respiration of its leaves returns from an increased rate to its normal status more quickly after water again has been supplied.

TABLE IV

EFFECT OF SOIL MOISTURE ON RESPIRATION OF McINTOSH APPLE LEAVES. EXPERIMENT IV

DATE, 1939	APPARENT VALUES FOR RESPIRATION IN CO_2/100 CM^3/HR.		EXPECTED RATE OF THE TEST TREES*
	TEST	CHECK	
	mg.	*mg.*	%
May 4	2.9	4.5	.....
5†	1.7	2.7	.....
6	2.6	4.2	.....
Average	2.4	3.8	158.3
7	3.0	5.7	120.0
8	2.2	4.3	123.4
9	4.0	8.5	134.2
10‡	1.7	3.5	130.0
11	2.2	3.7	106.3

* See table I for method of calculation.
† Test tree received last watering evening of May 5.
‡ Test tree watered to field capacity evening of May 10.

FIELD EXPERIMENTS[3]

During the summer of 1938 three experiments were carried out in a manner similar to those in the control chamber with the exception that the trees were placed outside the greenhouse where they were exposed to environmental conditions typical of central Ohio. The data are presented in figure 3 for one experiment which we considered, in general, almost identical with results obtained in the other two tests. Before it was evident that small variations in soil moisture had definite effects on leaf metabolism (as has been pointed out in the chamber experiments), it was decided to water the control plants in these tests only at intervals of 2 to 4 days when the soil appeared dry on the surface. The interval of watering obviously depended upon whether days were cloudy or sunny. This probably accounts for the fact that the relationship in photosynthesis between the two trees held practically constant for about three days after the dry period began (July 12) when the control tree also did not receive water for two days. Daily records show that water was applied to the control tree the mornings of July 12, 14, and the late afternoon of July 15 during the early part of the test period. From the afternoon of July 14 to the morning of July 18 the drying plant showed a definite relative increase (as much as 70 per cent., July 15) in carbon dioxide assimilation, which is similar to the behavior of drying plants in the chamber experiments. Beginning with the morning of July 19 there

[3] The authors appreciate the assistance of Mr. VERNON PATTERSON, senior in pomology, in performing the routine chemical analyses of the solutions.

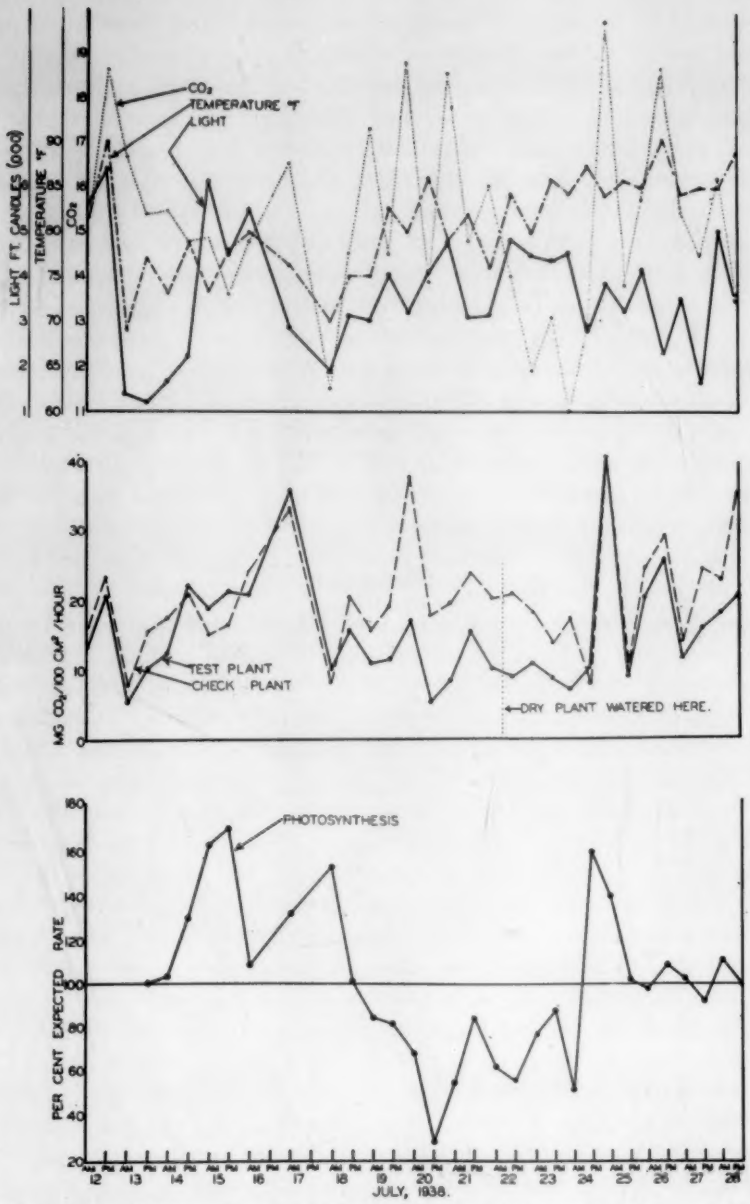

FIG. 3. The influence of soil moisture on apparent photosynthesis of apple leaves under central Ohio weather conditions in July. The test plant received water on July 12 and again on July 22 at 10: 50 A.M. In the lower part of the figure the relationship in photosynthesis between the test and check trees on July 12 and 13 was taken as a standard for comparison of leaf activity on the remaining days.

was a gradual decrease in photosynthesis as the plant continued drying. During this period the weather was generally cool and often cloudy which afforded ideal conditions for a very gradual drying of the plant, thus avoiding any sudden scorching of the leaf tissues. The leaves on the test tree showed no signs of wilting until late afternoon of July 19 when slight wilting on the lower leaves occurred; this again stresses the fact that there may be a marked reduction in photosynthesis by apple leaves before wilting is visible to the eye. On the morning of July 20 the test tree had recovered, but by mid-afternoon two lower leaves were scorched at the tip and margins and the lower leaves, in general, were badly wilted. Again, early in the morning of July 21, the plant had shown noticeable recovery overnight, but by late afternoon several more leaves were scorched and the entire plant was very definitely wilted. At 10:50 A.M., July 22 the dry plant was thoroughly watered but did not recover until late afternoon. The morning of July 23 it appeared to be fully turgid, and according to figure 3 had recovered in photosynthesis in about three days. It then was allowed to begin drying again when the experiment was discontinued.

Discussion

The data in experiments I, II, and III obtained in the environment-control chamber and the data obtained in the field (fig. 3) have shown a consistent increase in photosynthesis when the moisture in a relatively heavy type of soil fell below its field capacity. In an earlier report by HEINICKE and CHILDERS (**11**) similar results were noted and it was suggested that "the optimum soil moisture for photosynthesis of apple trees may be below the field capacity of the soil, or certainly, below a degree of saturation which persists for a day or so after watering." This would seem reasonable to expect in view of the numerous reports (**2, 15, 21**) which stress the importance of good soil aeration as it influences root and top growth. From the standpoint of moisture content of the leaves, BRILLIANT (**4**) has shown that photosynthesis is at its maximum when leaf moisture was reduced 5 to 15 per cent. in *Hedera helix* and *Impatiens parviflora*. It may also be noted in experiment II (90° F.) that transpiration showed a decrease of 15 per cent. at a time when there was a 6 per cent. increase in photosynthesis. It is probable that if transpiration tests had been made at the lower temperature of 80° F. (experiment I) when the period of increase in photosynthesis extended over a four-day period, the above relationship between photosynthesis and transpiration would have been more pronounced.

It has been stated by MILLER (**18**) that water plays both a direct and indirect rôle in photosynthesis: directly, by entering into chemical combination with carbon dioxide to form carbohydrates; and indirectly, by governing the opening of the stomata which, in turn, governs the intake of carbon

dioxide. In case of moderate water shortage to apple leaves, however, MAGNESS (**17**) states that there is so much water present in the leaf, even at time of stomatal closing, that it seems doubtful if a deficiency of water is a direct factor in reduced photosynthesis. In our experiments it has been difficult to establish a definite relationship between photosynthesis and stomatal opening at the time water becomes limiting; in several observations made by the junior author at Ithaca, N. Y., it appeared that there was a drop in carbon dioxide assimilation about the same time, or shortly after closing of the stomata was evident, due to water shortage. It does seem fairly clear, however, that under moderate weather conditions, there may be a 5 to 15 per cent. reduction in transpiration before stomata begin to show the effects of low water supply and before photosynthesis begins to show a definite decline (experiment II). MAGNESS (**17**) also found that moisture supply in the apple leaf may be reduced to an appreciable extent before stomata begin closing, "in some tests being approximately 5 to 7 per cent. higher at the time of stomatal opening than at the time of stomatal closing." He further states that under orchard conditions the early closing of stomata has proved to be about the best indication of the beginning of water shortage. Stomata, in most cases, were more sensitive to water deficiency than was the growth rate of the fruit.

With the type of soil used here (moisture equivalent of 38.4 per cent.; wilting coefficient of 15.55 per cent.) it is clear that there may be marked reductions in photosynthesis and transpiration and an increase in respiration before wilting in the apple is apparent. The length of time before wilting that these reductions may take place is dependent obviously upon the evaporating power of the air (experiments I (80°), II (90°), and III (100° F.)) and the mass of soil about the roots as well as the soil structure. The results presented here are in accord with those of LEWIS, WORK, and ALDRICH (**16**) who report in the case of heavy soils at Medford, Oregon, that fruit and tree growth were reduced significantly before soil about the majority of the roots of pear trees reached the wilting point. They state that fruit growth was reduced whenever soil moisture content fell below 70 per cent. of the available capacity. In case of the lighter soils (wilting coefficient of 8 to 10 per cent.) in California, however, HENDRICKSON and VEIHMEYER (**12**) have pointed out that fruit and tree growth were not reduced if the soil moisture remained about one per cent. or more above the wilting coefficient. HOWLETT (**13**) also states that with the Wooster Silt Loam (wilting coefficient of 7.5 to 8.0 per cent.) he was unable to detect any significant reduction in rate of fruit growth in the apple as long as the moisture was above the wilting coefficient.

It is generally considered uncertain that stomata are tightly closed even though they appear so under the microscope; it is interesting, however, to

note that on several occasions in these experiments we have obtained fairly high rates of photosynthesis when the stomata appeared to be completely closed. In his work with corn, SAYRE (**19**) has pointed out from considerable experience in the field where the "ultrapak" microscope was employed that "there is no close relation between stomata and photosynthesis in corn and that maximum photosynthesis can go on in the absence of open stomata." BERNBECK (**1**) also found that stomatal opening and photosynthesis operate independently under conditions of moderate water deficiency.

The time required for a wilted plant to recover to normal leaf activity after it has been watered is correlated, as one would expect, with the severity and length of the drought period [experiments II and III, and in the field (fig. 3)]. In experiment II, when the test plant was allowed to remain wilted for several days before watering, transpiration returned to approximately normal within 4 days while respiration and photosynthesis did not show complete recovery until the sixth and seventh day. In experiment III, where the plant was allowed to wilt only a single day, photosynthesis, transpiration, and respiration recovered within a day or two after the watering. Test plants in the field (fig. 3) were allowed to remain wilted for about two days and until several of the lower leaves on the shoots showed scorching and abscission before they were supplied with water. The time required for photosynthesis to recover was about two days. It should be pointed out that these reductions in leaf activity which persisted after water was supplied to the wilted plants, occurred even though the plants regained full turgidity within a few hours after watering. ILJIN (**14**) who worked with *Bidens tripartita* and *Phlomis pungens* also found that photosynthesis did not recover as soon as the wilted plants regained full turgidity but continued to show reductions of 29 per cent. 16 hours after recovery of turgor.

It is the opinion of YUNCKER (**22**) and others that when water supply to plants is reduced, the rate of respiration is likewise reduced; this does not seem to be the case in results presented here. In every case where respiration determinations were made on drying plants there was a relatively greater amount of carbon dioxide evolved from the drying leaves until the plant was watered, after which respiration gradually recovered to its normal relationship with the control tree. This is in line with the work of SMITH (**20**) who removed water from the leaves of snowdrop, stem tip of Tropaeolum, and young stems of Asparagus by means of a vacuum desiccator and showed that there was an increase in respiration proportional to the amount of water lost until 30 per cent. of the water had been removed. Respiration remained about the same when water was further diminished 50 to 60 per cent. From the data in tables II, III, and IV it is evident, also, that respiration of apple leaves assumed a more or less level status after the first two- or three-day climb, then dropped back upon addition of water.

In figures 1 and 3 it is evident that when data from the field are expressed in terms of percentage, the trend in photosynthesis is not so smoothly plotted as for data obtained in the environment-control chamber. For example, in figure 3, one would logically expect a greater reduction in assimilation of the drying plant on July 21 than on the previous day, July 20, because the soil, if anything, would be drier on the second day. The reverse, however, was true: there was a 66 per cent. reduction in photosynthesis on July 20 and only a 16 per cent. reduction on July 21. This and other unexpected variations in the photosynthetic relationship between the control and the test trees in the field might be explained by the wide variations in light, temperature, and other uncontrollable factors.

Summary

1. Determinations of apparent photosynthesis, transpiration and, in some cases, respiration were made on small apple trees while the soil in which they were growing gradually dried to the wilting percentage, after which it was watered to field capacity. The reference trees were watered at regular intervals. Experiments were performed both in the field and in a large insulated chamber where light was held constant at an average of about 4000 foot candles for all leaves concerned, humidity at about 30 per cent., and temperature at levels of 80°, 90° or 100° Fahrenheit.

2. The data show that an increase in apparent photosynthesis was associated with a slight decrease in soil moisture below the field capacity of the dark, fairly heavy soil employed in these experiments. Under temperature conditions of 100° F. this period of increase in photosynthesis was shorter (1 to 2 days) than when the temperature was 80° F. (2 to 4 days); this was probably due to the more rapid drying of the soil and the greater transpiration rate at the higher temperature.

3. At a temperature of 80° F. the first reduction in apparent photosynthesis occurred the fifth day after the last watering; at temperatures of 90° and 100° F. the first reduction in assimilation occurred on the third day. Ordinarily, under conditions of a gradually drying soil, there was a reduction in the rate of transpiration for at least one day before there was a reduction in photosynthesis. If evaporation conditions were low, this reduction in transpiration sometimes extended over a longer period of time before photosynthesis showed a definite decrease.

4. Before wilting was evident, there were marked reductions in apparent photosynthesis and transpiration, and an increase in respiration; in one case there was a 55 per cent. reduction in photosynthesis, a 65 per cent. reduction in transpiration, and a 62 per cent. increase in respiration. Stomata at this time appeared to be completely closed.

5. On several occasions fairly high rates of photosynthesis were obtained when the stomata appeared to be closed.

6. When the plants showed definite wilting, and the soil moisture was approximately at the wilting percentage, there was an 87 per cent. reduction in both photosynthesis and transpiration. It was not uncommon for wilted apple leaves to absorb from one to ten or more milligrams of carbon dioxide per 100 square centimeters of leaf surface per hour.

7. When water was applied to the soil in which wilted apple trees were growing, the leaves usually attained turgidity within three to five hours, depending upon their degree of wilting. They did not, however, recover to their original relationship with the controls in photosynthesis and respiration before two to seven days after the watering. Transpiration usually recovered about the same time as photosynthesis or slightly earlier.

8. The general trend of photosynthesis for the test plants was approximately the same in the field as in the environment-control chamber.

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LITERATURE CITED

1. BERNBECK, O. Wind und Pflanze. Flora **117**: 513–525. 1924.
2. BOYNTON, D., DEVILLIERS, J., and REUTHER, W. Are there critical oxygen concentrations for the different phases of root activity? Science n.s. **88**: 569–570. 1938.
3. ————, and SAVAGE, E. F. Soils in relation to fruit growing. Part XIII. Seasonal fluctuations of soil moisture in some important New York orchard soil types. Cornell Bull. 706. 1938.
4. BRILLIANT, B. Le teneur en eau dans les feuilles et l'energie assimilatrice. Compt Rend. Acad. Sci. (Paris) **178**: 2122–2125. 1924.
5. CHILDERS, N. F., KIPLINGER, D. C., and BRODY, H. W. A device for rapid rinsing of absorption towers used in gas analysis studies. Science n.s. **90**: 447–448. 1939.
6. ————, and BRODY, H. W. An environment-control chamber for study of photosynthesis, respiration and transpiration of horticultural plants. Proc. Amer. Soc. Hort. Sci. **37**: 384–390. 1939.
7. ————, and SCHNEIDER, G. W. The effect of water supply on the rate of photosynthesis, transpiration and respiration of apple leaves. (Abstract) Proc. Amer. Soc. Hort. Sci. **37**: 365. 1939.
8. DASTUR, R. H. The relation between water content and photosynthesis. Ann. Bot. **39**: 769–786. 1925.
9. HEINICKE, A. J. A special air chamber for studying photosynthesis under natural conditions. Science n.s. **77**: 516–517. 1933.
10. ————, and CHILDERS, N. F. The influence of water deficiency in photosynthesis and transpiration of apple leaves. Proc. Amer. Soc. Hort. Sci. **33**: 155–159. 1936.

11. ————————, and HOFFMAN, M. B. An apparatus for determining absorption of carbon dioxide of leaves under natural conditions. Science n.s. **77**: 55–58. 1933.
12. HENDRICKSON, A. H., and VEIHMEYER, F. J. Irrigation experiments with prunes. California Agr. Exp. Sta. Bull. 573. 1934.
13. HOWLETT, F. S. Relation of soil moisture content to apple tree growth and fruiting. Ohio Agr. Exp. Sta. Cir. no. 54. 26–28. 1938.
14. ILJIN, W. S. Der Einfluss des Wassermangels auf die Kohlenstoff-assimilation durch die Pflanzen. Flora **116**: 360–378. 1923.
15. KRAMER, P. J. Causes of decreased absorption of water by plants in poorly aerated media. Amer. Jour. Bot. **27**: 216–220. 1940.
16. LEWIS, M. R., WORK, R. A., and ALDRICH, W. W. Studies of the irrigation of pear orchards in heavy soils near Medford, Oregon. U. S. Dept. Agr. Tech. Bull. 432. 1934.
17. MAGNESS, J. R., DEGMAN, E. S., and FURR, J. R. Soil moisture and irrigation studies in eastern apple orchards. U. S. Dept. Agr. Tech. Bull. 491. 1935.
18. MILLER, E. C. Plant physiology. Chap. VIII: 598–599, second edition. McGraw-Hill Publishing Co., New York. 1938.
19. SAYRE, J. D. Corn stomata. Ohio Agr. Exp. Sta. Mimeo. (Presented before graduates and faculty of Plant Institute, Ohio State University.) 1939.
20. SMITH, A. M. The respiration of partly dried plant organs. Exp. Sta. Record **36**: 824. 1917.
21. TUKEY, H. B., and BRASE, K. D. Studies of top and root growth of young apple trees in soil and peat-soil mixtures of varying moisture contents. Proc. Amer. Soc. Hort. Sci. **36**: 18–27. 1938.
22. YUNCKER, T. G. A study of the relation of soil moisture to transpiration and photosynthesis in the corn plant. Plant World **19**: 151–161. 1916.

FACTORS AFFECTING ROOT FORMATION OF *PHASEOLUS VULGARIS*

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(WITH THREE FIGURES)

The successful rooting of leafy cuttings involves three physiologically distinct processes: initiation of root primordia; development of true roots from these initials; and maintenance of the cutting as an excised organ during the rooting.

The first of these, where it does not take place spontaneously, can be brought about in many plants by treatment with auxin. The second (outgrowth of the roots) involves the formation of cell-wall and protoplasm; it therefore requires the supply of carbohydrates and nitrogenous materials, together with such special substances as may be needed for root growth. Normally all of these materials would be supplied from the leaves or from storage in the stem. Too little attention has been paid up to now to the third process, the maintenance of the cutting, which, in view of the special conditions that obtain when leaves are severed from the plant, is of great importance. Thus, as pointed out by CHIBNALL (**4**), the detached leaf of many plants, including Phaseolus, undergoes a rapid and extensive proteolysis. The present experiments were, therefore, designed to shed some light on the second and third of these processes.

It was our intention to study the influence of nutrition, especially nitrogen nutrition, and other external chemical factors on the rooting and maintenance of leafy herbaceous material.

Since internal factors must also be considered in such a study, attention was paid to the rôle of auxin and also to the separate rôles of the leaves and the stem.

Materials and methods

Phaseolus vulgaris, the red kidney bean, was used throughout. The plants were raised in flats in the greenhouse and used at the age of about two weeks. The first simple leaf with its petiole (referred to as "leaf" throughout the text) was used in the majority of the experiments; isolated internodes, hypocotyls, and stem sections with attached leaves were also employed for comparison.

All solutions were made up with distilled water. Treatments were basal, and applied by immersion to a depth of about 2 cm. in the solution tested. The cuttings were supported by cotton plugs inserted into holes in thin wood covers, in such a manner that their bases dipped into beakers containing either water or the solution to be tested; the beakers were held in a wooden frame. In this way the bases were kept in darkness throughout

the experiment. The tops received the ordinary light conditions of the greenhouse. This was supplemented in the evening with a "sunlight" arc lamp to give a total constant day-length of fourteen hours since many experiments were carried out from November, 1939, to March, 1940. Each experiment lasted 15 to 20 days from the time of cutting. During the first twenty-four hours the cuttings were kept in auxin or water, the next twenty-four hours in the tested solution, and the following thirteen to eighteen days in distilled water.

Indole-3-acetic acid was employed as the auxin throughout; adenine was used as sulphate, choline and guanine as hydrochlorides. The weights of these substances given below, however, refer to the actual weight of base present.

Experimental results

The influence of auxin

The effects of treating the base of the petiole with indole-acetic acid of various concentrations are summarized in table I. The data are taken

TABLE I

Influence of treatment with indole-acetic acid for 24 hours on the survival and rooting of *Phaseolus vulgaris* cuttings*

Indole-acetic acid used per liter	Cuttings alive		Cuttings rooted		Average number of roots per rooted cutting	
	Water control	Auxin treated	Water control	Auxin treated	Water control	Auxin treated
mg.	%	%	%	%		
40	76	27	70	27	....	....
10 (mean of 2 series)	75	46	68	46	....	....
2 (mean of 2 series)	78	64	67	60	....	....
0.5	25	29	4	29	10	40
0.1	61	46	54	40	63	44
0.025 (mean of 4 series)	80	69	41	37	23	57
0.025 (internode attached)	100	100	75	92	23	24

* All figures but the last refer to leaf cuttings; the last series have one complete internode attached. Results after 15 days in greenhouse.

from a number of separate experiments, each of which, however, included its own water controls.

It will be seen that the higher auxin concentrations caused a serious increase in mortality; even very low concentrations, in all but two of the experiments with the leaf-cuttings, somewhat reduced the number surviving. In only one of these two cases (0.5 mg. per liter) was there a significant increase in the percentage rooting.

On the other hand, practically all of the surviving cuttings, treated with auxin at 0.025 mg. per liter or higher, rooted; in the water controls, 10 to 80 per cent., or an average of about one-third of those surviving, did not root in the fifteen days. This may be seen by comparing the percentage alive with the percentage rooted in the same experiment. Further, in all but one of the seven experiments in which the number of roots was counted, auxin treatment greatly increased the number of roots per rooted cutting. This effect is not greatly dependent on auxin concentration. It is strongest in the two cases where rooting in water was poor. Examples of large increases in the number of roots formed may also be seen in tables III, VI and VII. To put it briefly, those cuttings which survive the auxin treatment show an increased root formation, but some cuttings are damaged even at concentrations as low as 1/40 mg. per liter. It is evident that this material has an unusually high sensitivity, to both the root-initiating and the toxic action of indole-acetic acid.

The notably lower mortality and higher rooting ability of the cuttings with attached stem is discussed in the next section.

Influence of the leaf and stem

In preliminary experiments it was found that the maintenance of single-leaf cuttings in water or nutrient solution depended on the amount of stem attached. With Phaseolus, which wilts easily, this phenomenon is very striking. The following figures show the percentage of cuttings which were evidently alive and not wilted when kept with their bases in distilled water for one week in the laboratory:

Cutting	Percentage survival
Leaf plus petiole (cut at A, see figure 1)	5
Leaf plus petiole plus node (cut at B)	22
Leaf plus petiole plus node plus half the next lower internode (cut at C)	30
Leaf plus petiole plus node plus whole next lower internode (cut at D)	67
Leaf plus petiole plus node plus whole next lower internode plus next lower node (cut at E)	78

Another comparison, with somewhat younger plants, done in the greenhouse, is presented in table II. Though the survival is higher and thus more normal, the beneficial influence of the added stem section is still clear. Since the internode by itself does not survive appreciably (column 4) it is the combination of leaf with stem tissue which is most viable. The results in nutrient solution are similar to those in water, so that the effect cannot be ascribed to simple nutritional deficiency.

Root formation shows the same response as survival. The effect of an attached internode is shown in table III. The cuttings were first treated

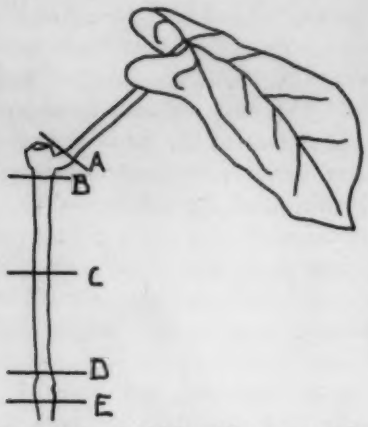

FIG. 1. Sketch showing points at which cuttings were taken.

TABLE II

SURVIVAL OF VARIOUS TYPES OF PHASEOLUS CUTTINGS AFTER 15 DAYS IN THE GREENHOUSE, IN WATER OR IN NUTRIENT*

	PERCENTAGE OF CUTTINGS ALIVE				
	LEAF ONLY; (CUT AT A) MEAN OF 3 EXPTS.	LEAF PLUS NODE; (CUT AT B) 1 EXPT.	LEAF PLUS NODE PLUS INTERNODE; (CUT AT D) 1 EXPT.	ISOLATED (LEAFLESS) INTERNODE; MEAN OF 2 EXPTS.	ISOLATED HYPOCOTYL; MEAN OF 2 EXPTS.
	%	%	%	%	%
In water	65	70	85	2	17
In nutrient solution	67	90	90	2	12

* Hoagland's solution, quarter strength. For points at which cutting was made, see figure 1.

TABLE III

ROOTING AND MORTALITY AFTER 16 DAYS IN WATER IN THE GREENHOUSE. AUXIN TREATMENT 0.025 MG. PER LITER

	PERCENTAGE ROOTED		NUMBER OF ROOTS PER ROOTED CUTTING		PERCENTAGE ALIVE	
FIRST 24 HOURS IN:	WATER	AUXIN	WATER	AUXIN	WATER	AUXIN
	%	%			%	%
Leaf (cut at A)	23	8	3	47	92	80
Leaf plus internode (cut at D)	69	92	21	22	100	100

basally with water or auxin (0.025 mg. per liter) for 24 hours, then kept with their bases in water for 16 days and roots counted. When the in-

ternode is absent, auxin increases the number of roots per rooted cutting. It also evidently causes some toxicity, as shown by reduced percentage of rooting and increased mortality. When the internode is attached this toxicity disappears and at the same time the percentage of cuttings rooted, either with or without auxin, is greatly increased and survival becomes complete.

The explanation for this influence of attached stem sections in maintaining the cutting alive cannot as yet be given, but it seems highly probable that it is due to a contribution of some organic substance, or substances, by the stem.

After-treatment of the cuttings

Mineral nutrients.—In maintaining cuttings in solution it was first necessary to know whether or not they required aeration. In comparative experiments with leaves, internodes, and hypocotyls the influence of aerating the water or nutrient solution was found to be negligible. In most instances, indeed, the stream of air so promoted the growth of microörganisms that the cuttings became infected. Aeration was therefore not adopted.

The effect of providing nutrients was tested in a number of experiments. Hoagland's solution (7) at one quarter strength was found satisfactory. It maintained the roots in white and active condition, as compared to the evident browning after 15 days in water. On the other hand the solution, if anything, inhibited root initiation. In one experiment leaves, with and without auxin treatment, rooted 42 and 45 per cent., respectively, in nutrient solution; those kept in water rooted 55 and 50 per cent., respectively.

TABLE IV

Influence of nutrient salts. Auxin 0.025 mg. per liter

First 24 hours	Nutrient solution second 24 hours	Cuttings rooted	Mean number of roots per rooted cutting	Total number of roots on 30 cuttings
		%		
Water	Water	60	27	520
Auxin		60	29	
Water	$Ca(NO_3)_2$ 200 mg./l.	47	26	407
Auxin		47	35	
Water	KH_2PO_4 20 mg./l.	60	45	487
Auxin		27	23	
Water	KH_2PO_4 200 mg./l.	27	13	369
Auxin		53	39	
Water	KNO_3 50 mg./l.	47	82	827
Auxin		73	23	
Water	KNO_3 500 mg./l.	67	21	785
Auxin		97	41	

This slight retarding effect of the complete nutrient solution is, however, in sharp contrast with the behavior of the individual salts separately. Rooting was markedly inhibited by $MgSO_4$ and by $Ca(NO_3)_2$ at the same concentration as in the nutrient solution; but strongly promoted by KNO_3 and in a few experiments weakly by KH_2PO_4. The most powerful inhibition was exerted by $MgSO_4$, which at M/3000 (82 mg. per liter) completely prevented any rooting of leaves and increased the mortality from 13 to 33 per cent. A comparison of the other salts is given in table IV. The beneficial influence of KNO_3 at relatively high concentrations can best be seen from the total number of roots in the last column.

The data in table IV, showing the increased root formation when cuttings were supplied with KNO_3, were extended by comparison with another source of available nitrogen, namely ammonium sulphate. Table V shows that

TABLE V

COMPARISON OF NITRATE WITH AMMONIUM. INDOLE-ACETIC ACID 0.5 MG. PER LITER. RESULTS AFTER 15 DAYS IN GREENHOUSE

1ST 24 HOURS IN	SECOND 24 HOURS		ROOTED	TOTAL NUMBER OF ROOTS ON 24 CUTTINGS
			%	
Water	Water		4	100
Auxin			28	202
Water	KNO_3	20 mg./l.	8	160
Auxin			25	270
Water	KNO_3	200 mg./l.	25	280
Auxin			33	270
Water	$(NH_4)_2SO_4$	2 mg./l.	8	35
Auxin			8	35
Water	$(NH_4)_2SO_4$	20 mg./l.	0	0
Auxin			0	0

even at 2 mg. per liter (M/66000) rooting is strongly inhibited, while 20 mg. per liter and all higher concentrations completely prevent all rooting. KNO_3, on the other hand, is again effective.

ORGANIC NITROGEN.—Organic nitrogenous compounds, especially those known to be present in leaves, are of particular interest in this connection. The analyses of VICKERY (8) on alfalfa show that, apart from amino-acids, the most important nitrogenous constituents of the leaves are asparagine, adenine, stachydrin, and choline. Since the bean is also a legume, these substances were considered the most important to study. Stachydrin was, however, omitted, and instead some other purines and nicotinic acid were used.

The results showed considerable variation from experiment to experi-

ment, and will therefore be given in abbreviated form. The controls themselves varied markedly, perhaps due to small differences in humidity and lighting which are unavoidable under greenhouse conditions. There is also some evidence that when cuttings are taken late in the day their subsequent survival is impaired. In each of the experiments, however, the cuttings for all treatments were taken at one time. Two additional reasons add to the difficulty of drawing conclusions. One is that some substances showed effects mainly when the cuttings had been pre-treated with auxin; *i.e.*, they enhanced the effectiveness of the auxin treatment, while others were more effective when auxin had not been used. The other is that in some instances the percentage of cuttings which rooted was increased, while the total number of roots formed on all the cuttings was not. The opposite also occurred. For example, the best single effect on root number was obtained with guanine 20 mg. per liter, which gave 583 roots per 15 leaf cuttings against 113 in the controls, *i.e.*, an increase of 31 roots per cutting. The highest increase in percentage rooting of leaf cuttings was, however, in one of the experiments with nicotinic acid 20 mg. per liter, which gave 67 per cent. rooting as against only 20 per cent. in the controls. In spite of these uncertainties, the great effectiveness of adenine and asparagine in promoting rooting may be regarded as fully established, while that of the other substances tested is at least highly probable.

Adenine gave the most consistent results. Out of 6 experiments, each one including both water and auxin pre-treatment (12 comparisons), adenine increased the percentage rooting and the total number of roots in every case. The data are summarized in figure 2, which shows the increases above the controls for each of three concentrations used. Concentrations below 50 mg. per liter showed no consistent effectiveness. Apparently the actual concentration which causes the best effect depends on whether auxin was used or not, the higher concentrations being more effective with auxin pretreatment, the lower without. It is tempting to ascribe this to a "limiting factor" effect such as is discussed by WENT and THIMANN (9); the data obtained with other substances, however, do not bear this out.

The effects of adenine were of good magnitude. In one experiment 4 per cent. rooted in water controls and 42 per cent. in those treated with adenine 50 mg. per liter; in another 38 per cent. rooted in auxin controls and 77 per cent. when auxin was followed by adenine 200 mg. per liter. Also, in spite of the variation between control values in different experiments, the results are statistically fully significant. The mean percentage rooting of all *controls* in the adenine experiments (both water and auxin treated) was 22.5 ± 0.8, while the mean percentage rooting of all *adenine-treated* cuttings (both water and auxin pretreated) was 41.8 ± 0.5. As to root number, the controls averaged, per 15 cuttings, 142 ± 8 roots, those

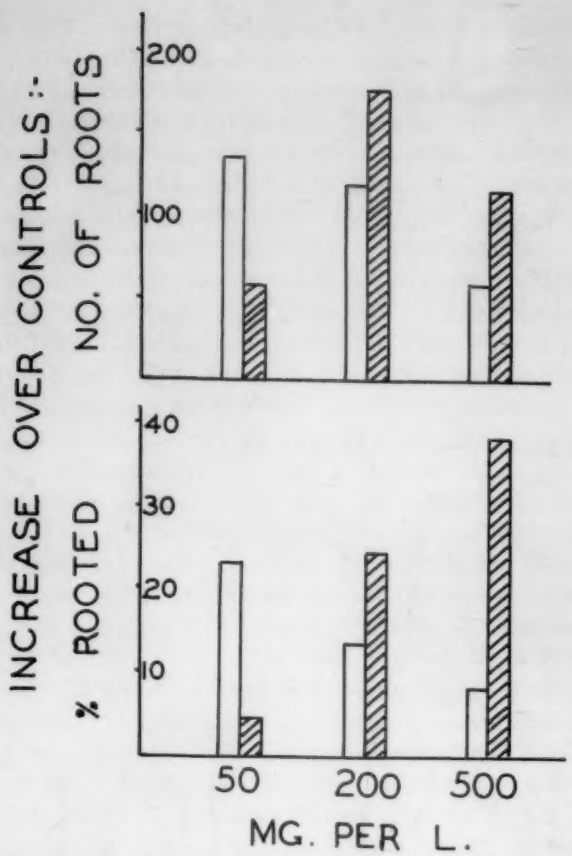

FIG. 2. The effect of adenine as after-treatment.

Ordinate: increase in root number and in rooting percentage as a result of adenine treatment.

Abscissa: concentration of adenine.

Shaded columns: cuttings pretreated with auxin.

White columns: no auxin.

Data for 50 mg. per liter the mean of two experiments; those for 200 mg. per liter the mean of three.

treated with adenine 252 ± 7. Examples of single experiments are also included in tables VII, VIII, and IX.

The higher rooting of cuttings with attached internode (such as were used for table III) apparently precludes any further increase by adenine. One such experiment was carried out, but both the controls and those given adenine rooted 85 per cent. and both averaged 18 roots per cutting.

The results of two similar experiments with asparagine are given in table VI. High concentrations were not practicable on account of infection.

TABLE VI

INFLUENCE OF ASPARAGINE (5 MG. PER LITER)*

FIRST 24 HOURS	SECOND 24 HOURS			
	WATER		ASPARAGINE	
	PERCENTAGE ROOTING	TOTAL NUMBER OF ROOTS ON 15 CUTTINGS	PERCENTAGE ROOTING	TOTAL NUMBER OF ROOTS ON 15 CUTTINGS
	%		%	
Water	8	8	13	45
Auxin	16	150	21	112
Water	54	509	69	726
Auxin	38	254	62	516

* Auxin: indole-acetic acid 0.1 mg. per liter. Results after 15 days in greenhouse. Two separate experiments, each with water and auxin treatment.

The optimum concentration has evidently been covered, however, since 50 mg. per liter was less effective than 5 mg. per liter, which was used for both series of table VI. Seven of the eight comparisons show real increases. The mean increase in percentage rooting caused by asparagine is 12 ± 1.3; the mean increase in root number is 120 ± 20, so that the data are fully significant. On the whole, asparagine 5 mg. per liter was nearly as effective as adenine.

Two experiments with guanine are summarized in figure 3. The percentage of cuttings rooting was increased markedly in five out of the six comparisons, but the total number of roots was increased only when auxin had not been supplied. The increases were, however, very large.

Results with uracil, 0.1 and 5 mg. per liter, were somewhat erratic, but

TABLE VII

INFLUENCE OF YEAST NUCLEIC ACID AND OF ADENINE*

FIRST 24 HOURS	SECOND 24 HOURS				TYPE OF CUTTING
	WATER	NUCLEIC ACID 200 MG./L.	NUCLEIC ACID 500 MG./L.	ADENINE 500 MG./L.	
Water	9	0	13	67	Leaf
Auxin	55	20	98	171	
Water	198	175	.....	.....	Leaf
Auxin	261	520	.....	.....	
Water	255	315	375	.....	Leaf and one internode
Auxin	300	285	420	.....	

* All data the total number of roots on 15 cuttings. Auxin: indole-acetic acid 0.025 mg. per liter. Results after 15 days in greenhouse.

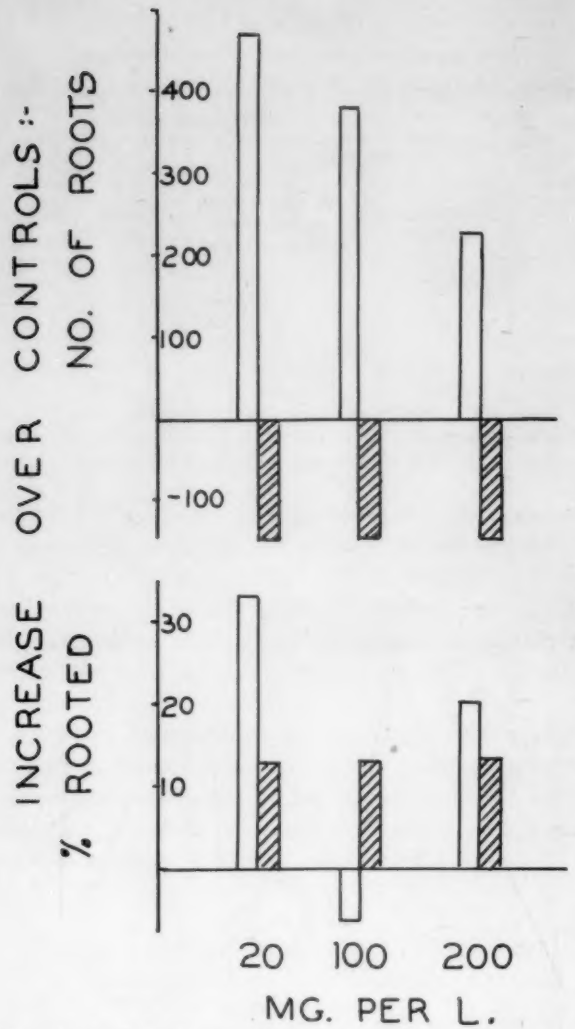

FIG. 3. The effect of guanine as after-treatment. Legends as for figure 2. Each concentration one experiment only. The number of roots is increased only when auxin is not used.

there was a marked increase in root number in the cuttings pretreated with auxin (504 on 15 controls, 771 on 15 uracil-treated).

It was thought that the beneficial effect of the purines might be combined with that of phosphate by administering nucleic acid. Yeast nucleic acid was therefore tested at 20 to 500 mg. per liter. The higher concentrations appeared to show a real increase in root number (table VII) but no increase in percentage rooting. Adenine in the same experiment was much more

effective. Cuttings with internode attached also showed a response to nucleic acid at 500 mg. per liter. Infection of the bases of the cuttings was, however, very troublesome at these high concentrations.

An experiment with choline is presented in table VIII. In the absence

TABLE VIII

INFLUENCE OF CHOLINE AND OF ADENINE. AUXIN 0.5 MG. PER LITER. RESULTS AFTER 15 DAYS IN GREENHOUSE

FIRST 24 HOURS	PERCENTAGE OF ROOTING			
	WATER	CHOLINE 5 MG./L.	CHOLINE 50 MG./L.	ADENINE 50 MG./L.
	%	%	%	%
Water	4	25	25	42
Auxin*	29	8	0	25
	TOTAL ROOTS ON 15 CUTTINGS			
Water	6	89	140	250
Auxin*	144	69	0	119

* The auxin data are included for the sake of completeness, although the concentration used was evidently too high.

TABLE IX

INFLUENCE OF ADENINE. AUXIN 0.1 MG. PER LITER. RESULTS AFTER 15 DAYS IN GREENHOUSE

FIRST 24 HOURS	PERCENTAGE OF ROOTING		
	WATER	ADENINE 50 MG./L.	ADENINE 200 MG./L.
Water	54	62	70
Auxin	40	46	77
	TOTAL ROOTS ON 15 CUTTINGS		
Water	509	535	831
Auxin	255	494	646

of auxin treatment, both total root number and percentage rooting are clearly increased, but the combination of choline with auxin pre-treatment seems to be very damaging. This may be due to the rather high auxin concentration used in this particular experiment. For comparison, the data for adenine (50 mg. per liter) obtained in the same experiment are included. They show the same tendency to fall where auxin was used, but they also show how large an effect can be exerted by adenine.

Nicotinic acid, at 4 to 100 mg. per liter, produced small increases in the percentage rooting and the number of roots formed, mainly when auxin was not supplied, but the response was variable and no certain conclusions can be drawn. It may also be noted that malate M/1000, was completely without effect. This is of interest since malic acid appears to function in the oat coleoptile as part of the auxin complex.

Discussion

The powerful influence of a section of stem, both in maintaining the cuttings and promoting rooting, shows that the stem exerts an important influence on the leaf. Because of the extensive proteolysis is initiated in leaves on their removal from the stem, even in light (**4**, ta 91), the inference may be made that the presence of the stem in some way prevents this proteolysis. Probably, therefore, maintenance of the attached leaf is a function of the degree to which proteolysis is prevented. In view of the clear effects exerted by nitrate and organic nitrogen in the present experiments, it is suggested that the stem contributes to the leaves an available form of nitrogen. Indeed, BOTH (**3**) has shown that nitrogenous material can be readily transported from a stem section into an attached leaf, provided only that the water-vapor tension is below saturation.

It is of interest that besides nitrate and asparagine, the purines, adenine especially, are so effective. The importance of adenine for the synthesis of nuclear material and of coenzymes, and the ease with which asparagine is metabolized in the plant, doubtless account for the fact that these two are the most effective substances of those tested. The activity of adenine on the expansion of leaf-blades has been reported by BONNER and HAAGEN-SMIT (**2**), and apparently in some plants the effect is exerted on vegetative growth generally (**1**). The range of active concentrations, however, in their continuous watering experiments is much lower than those effective in the present short-time treatments. More closely related, perhaps, are the interesting recent observations of DOAK (**5**), who found a variety of nitrogenous compounds, mostly amino-acids and purines, to promote rooting in cuttings of a Rhododendron. However, while asparagine was one of the most active substances he tested (in agreement with our data), adenine and guanine were much less so. The general point that small quantities of organic nitrogen enabled considerable rooting to occur remains clear in both cases.

In view of the effectiveness of nitrate, it is possible that the improvement in rooting of spruce cuttings caused by nutrient solution (**6**) is due to the nitrate in the solution, particularly since (as shown above) Ca and PO_4 have little influence, while $MgSO_4$ has even the opposite effect.

The usual methods of propagation by cuttings entail more or less com-

plete nitrogen starvation of the material. The present results suggest that, for some plants at least, this procedure might well be radically revised.

Summary

The rooting of isolated leaves of *Phaseolus vulgaris*, with or without attached stem sections, has been studied.

1. The survival of the cuttings after removal from the plant is greatly improved by presence of a portion of stem, and varies roughly with the amount of stem attached.

2. The material is extremely sensitive to auxin, a concentration of 0.025 mg. indole-acetic acid per liter being the highest that can be applied (for 24 hours) without producing injury. However, auxin treatment in nearly all experiments considerably increased the number of roots formed per rooted cutting.

3. Rooting is slightly depressed by a complete nutrient solution, or by the Ca or Mg salts composing it, but is strongly promoted by potassium nitrate. Ammonium sulphate, even at high dilution, is inhibitory. The growth and maintenance of roots already formed is, however, improved by the complete nutrient solution.

4. Of the organic nitrogen sources studied, adenine was the most effective, greatly increasing both the percentage of cuttings which rooted and the number of roots formed by each rooted cutting. Asparagine, guanine, choline, and uracil were also effective to a lesser extent, in approximately that order, while yeast nucleic acid and nicotinic acid showed slight but variable effects.

It is concluded that the nitrogen nutrition of the isolated leaf is of great importance in determining both its survival and its root formation.

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LITERATURE CITED

1. Bonner, D. M., and Bonner, J. On the influence of various growth factors on the growth of green plants. Amer. Jour. Bot. **27**: 38–42. 1940.
2. ————————, and Haagen-Smit, A. J. Leaf growth factors. II. The activity of pure substances in leaf growth. Proc. Nat. Acad. Sci. **25**: 184–188. 1939.
3. Both, M. P. Stoffwanderung in einfachen Systemen. Rec. Trav. Bot. Néerl. **34**: 2–59. 1937.

4. CHIBNALL, A. C. Protein metabolism in the plant. Yale University Press, New Haven, 1939.
5. DOAK, B. W. The effect of various nitrogenous compounds on rooting of Rhododendron cuttings with naphthalene-acetic acid. New Zealand Jour. Sci. Techn. **21**: 336A–343A. 1940.
6. GRACE, N. H. Vegetative propagation of conifers. II. Effects of nutrient solutions and phytohormone dusts on the rooting of Norway spruce cuttings. Canadian Jour. Res. **17**: 312–316. 1939.
7. HOAGLAND, D. R., and SNYDER, W. C. Nutrition of the strawberry plant under controlled conditions. Proc. Amer. Soc. Hort. Sci. **30**: 288–294. 1933.
8. VICKERY, H. B. Yearbook Carnegie Institution of Washington **24**: 349. 1925.
9. WENT, F. W., and THIMANN, K. V. Phytohormones. New York, 1937.

TRANSFORMATION OF SUGARS IN EXCISED BARLEY SHOOTS

R. M. McCready and W. Z. Hassid

(with two figures)

Introduction

Nelson and Auchincloss (**10**) working with potato tissue showed that sucrose is synthesized from glucose or fructose rather than from starch as has been supposed by some investigators. That such a conversion occurs in plant metabolism has also been shown by Virtanen and Nordlund (**14**) in red clover and wheat plants, and more recently by Hartt (**3**) in detached sugar cane leaves.

Since sucrose, consisting of a glucose and fructose molecule, can readily be synthesized from either of these monosaccharides, it may be assumed that such synthesis involves a preliminary conversion of each of these hexose sugars into the other. The fructose component of the sucrose molecule exists in the furanose form (unstable γ form with the five-membered ring) and is different from the six-membered fructose (pyranose configuration) from which it can be synthesized. The plant apparently also possesses a mechanism to render this transformation possible. The fact that sucrose is synthesized when plants are artificially supplied with glucose or fructose can serve indirectly in the study of transformation of other hexoses in the plant into these monosaccharides. If, for example, synthesis of sucrose is observed after supplying the plant with mannose, it could be assumed that a preliminary conversion of the latter into glucose and fructose must take place before the synthesis of sucrose occurs. The hexoses that are known to occur naturally in plants are: d-glucose, d-fructose, d-mannose, and d-galactose; of these the last two have not been detected in the free state and are found in plants only as units in polysaccharides. If, as it is generally assumed, some form of glucose is the first product of photosynthesis, the plant should possess a mechanism whereby this molecule is transformed into mannose or galactose before the synthesis of the corresponding polysaccharide can be possible.

In this investigation, using the infiltration method (**8**), the following compounds were artificially supplied to barley plants: d-glucose, d-fructose, d-mannose, d-galactose, sucrose, lactose, maltose, l-arabinose, d-xylose, mannitol, sorbitol, gluconic acid, pyruvic acid, and glyceric aldehyde. The increase of sucrose was observed after certain periods of time to determine the ability of the plant to transform these compounds into glucose or fructose and, if possible, to ascertain the mechanism of this transformation.

Experimental methods and results

It is well known that detached leaves or plants detached from their roots are capable of carrying on metabolic activities for a considerable length of time. KURSANOV (**8**) utilized this fact to study the synthetic and hydrolytic actions of invertase in living plant tissues by his "vacuum infiltration" method. This method was effectively used in our investigation of sugar transformations in barley plants. Barley plants were grown in half strength HOAGLAND's culture solution for about 3 weeks. The plants were cut above the roots (only the shoots were used) and 5-gm. samples of the fresh material were immersed into beakers containing 5 per cent. sugar solution. The beakers with contents were placed into a desiccator and evacuated to about 20 mm. of mercury for 5 minutes. After evolution of the air bubbles from the leaves had ceased, air was slowly re-admitted into the desiccator. The intercellular spaces were thereby replaced by the sugar solution. The plant material was then taken out of the beakers, and allowed to remain in the dark at about 22° C. in an atmosphere saturated with water vapor for a certain period of time. The material was then thoroughly washed with water, the soluble sugars extracted with boiling 80 per cent. alcohol and analyzed for reducing sugars by oxidation with ferricyanide and titration with ceric sulphate (**6, 7**) before and after hydrolysis with invertase. Sucrose was determined by the difference. Blank samples of 5 gm. fresh weight of the same plant material infiltrated with water were run simultaneously. In all cases the samples were run in duplicate. The results are expressed on the basis of fresh weight.

Preliminary experiments showed that barley samples infiltrated with 5 per cent. solutions of glucose, fructose, mannose, galactose, lactose, and maltose, and allowed to remain for several hours showed a considerable gain in sucrose over the blank samples infiltrated with water. This indicated synthesis of sucrose by the plant from these sugars. Since the synthesis was measured by difference in sucrose between the sugar and the water infiltrated samples, the production of sucrose in these experiments was subject to doubt. It should be borne in mind that besides synthesis of sucrose, other metabolic processes go on simultaneously in the plant. While synthesis of sucrose is taking place, glucose or fructose is being used up in respiration, and in order to maintain a proper state of equilibrium a certain amount of sucrose is hydrolyzed back into these monosaccharides. In the samples infiltrated with solutions of monosaccharides, there is an excess of hexose sugars available for respiration. The hydrolysis of sucrose into glucose and fructose will therefore tend to decrease, and the equilibrium represented by the equation: glucose + fructose $\rightleftarrows$ sucrose will be shifted to the right. In the water infiltrated samples the tendency of the sucrose to hydrolyze is greater, and the equilibrium will be shifted to the left. The

difference in sucrose content between the sugar infiltrated sample and the blank would therefore not represent the true value of the amount of sucrose synthesized. To avoid this difficulty a procedure was adopted to deplete the sucrose to a low level, by respiring the barley plants for a certain length of time previous to infiltration with the sugar solutions.

The tendency of sucrose to be depleted first during respiration, while the reducing sugars are maintained more or less at a constant level, is shown by the results in table I.

TABLE I

COMPARISON OF SUCROSE AND REDUCING SUGARS IN EXCISED BARLEY SHOOTS AFTER RESPIRATION FOR 24 AND 48 HOURS IN THE DARK AT 22° C.

SAMPLE	DESCRIPTION OF SAMPLE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	SUCROSE PER 5 GM. OF FRESH MATERIAL
		%	%	%	*mg.*
1	Initial untreated	0.87	1.10	0.22	11.0
2	Depleted (24 hours) in water	0.90	0.90	0.0	0.0
3	Depleted, then water infiltrated 24 hours	0.77	0.77	0.0	0.0
4	Depleted, then infiltrated 5% glucose 24 hours	1.09	1.43	0.32	16.0

The untreated sample, no. 1, analyzed immediately after harvesting, contained 0.87 per cent. reducing sugars and 0.22 per cent. sucrose or 11.0 mg. per 5 gm. of fresh weight. After the plants respired in the dark for 24 hours the reducing sugars remained at about the same order of magnitude, 0.9 per cent. but the sucrose was all depleted (no. 2 sample). When the respired sample, no. 3, was infiltrated with water and allowed to remain for another 24 hours, the reducing sugars came down to 0.77 per cent. A decrease in the reducing sugars could be observed only after all the sucrose was exhausted. Sample no. 4, which was infiltrated with 5 per cent. glucose after all the sucrose was depleted, synthesized 16.0 mg. of sucrose per 5 gm. of fresh weight in 24 hours and contained 1.09 per cent. reducing sugars.

Table II represents results of experiments with barley shoots which respired in the dark for 24 hours to a low level of sucrose of 1.0 mg. per 5 gm. of fresh weight. Samples of these plants were then infiltrated with 5 per cent. solutions of glucose, fructose, mannose, galactose, and arabinose and allowed to remain for 18 hours. As shown in the table, sucrose was formed from glucose, fructose, mannose, and galactose but not from arabinose. The relative rate of sucrose synthesis in the plant from these sugars with

TABLE II

SUCROSE SYNTHESIS IN SUCROSE DEPLETED BARLEY SHOOTS AFTER INFILTRATION WITH 5 PER CENT. SOLUTIONS OF VARIOUS MONOSACCHARIDES AND ALLOWED TO REMAIN FOR 18 HOURS AT 22° C.

SAMPLE	DESCRIPTION OF SAMPLE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	SUCROSE PER 5 GM. OF FRESH MATERIAL	RELATIVE RATE OF SUCROSE FORMATION
		%	%	%	mg.	
1	Initial untreated	0.86	1.14	0.27	13.5	.....
2	Depleted (24 hours) in water	0.78	0.80	0.02	1.0	.....
3	Depleted, then water infiltrated 24 hours	0.58	0.60	0.02	1.0	.....
4	Glucose infiltrated	0.50	0.97	0.45	22.5	100.0
5	Fructose infiltrated	0.50	0.83	0.31	15.5	67.5
6	Mannose infiltrated	1.64	1.89	0.24	12.0	51.2
7	Galactose infiltrated	1.73	1.88	0.14	7.0	27.9
8	Arabinose infiltrated	1.52	1.52	0.0	0.0	.....

respect to glucose was as follows: fructose, 67.5 per cent.; mannose, 51.3 per cent.; galactose, 27.9 per cent.

Figure 1 gives the results from 8 samples of barley plants which were infiltrated with 10 per cent. glucose solution, analyzed for sucrose immediately, and then the synthesis of sucrose followed at different intervals for 48 hours.

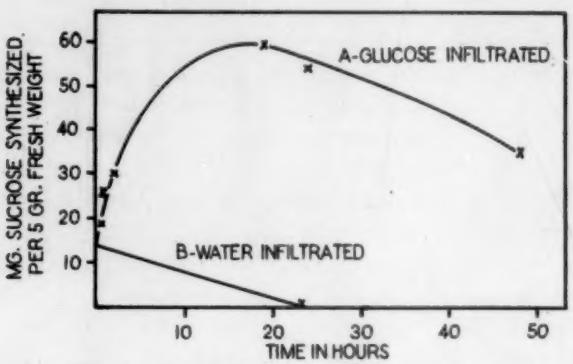

FIG. 1. Synthesis of sucrose from glucose.

As seen from curve A the maximum amount of sucrose was synthesized after about 18.5 hours (59 mg. per 5 gm.). From then on the amount of synthesis gradually diminished. Line B shows the depletion of sucrose. The entire content of sucrose was depleted from an equivalent sample of barley within 24 hours. Curve A therefore represents the synthesis of sucrose above the amount depleted during respiration.

The synthesis of sucrose from galactose is shown in figure 2.

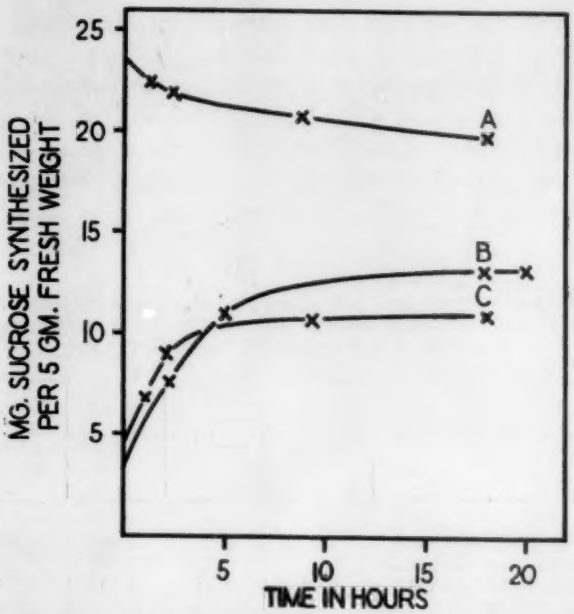

FIG. 2. Synthesis of sucrose from galactose during 20 hours. A, not depleted of sucrose before infiltration. B, depleted. C, recrystallized galactose and sample depleted before infiltration.

Galactose was infiltrated into one set of barley samples, originally containing 23.5 mg. per 5 gm. of fresh weight, analyzed immediately and the synthesis of sucrose followed for 18 hours. Since the rate of sucrose formation from galactose is not very great (shown in table II, 27.9 per cent. as from glucose) it did not exceed the hydrolysis which was apparently simultaneously taking place during respiration. Therefore, not a gain but rather a slight loss in sucrose could be observed, as shown by slightly downward curve A. In a second set of barley plants, however, in which the sucrose had been previously exhausted to a low level, a distinct gain of sucrose could be observed from galactose, as shown by curve B.

Since commercial galactose is usually made by hydrolysis of lactose, which consists of one molecule of galactose and one molecule of glucose, it was tested for purity with respect to glucose contamination. A fermentation test with a pure culture of yeast (*Torula monosa*) showed that the galactose contained almost 1 per cent. glucose. The commercial galactose was therefore purified by dissolving in water and fermenting the glucose and then crystallizing from alcohol. A fermentation test on this purified galactose showed no glucose contamination. Curve C shows sucrose formation when purified galactose was used. This eliminates the possibility that the sucrose could have been formed from the small amount of glucose found as an impurity and not from the galactose.

Table III shows the results of experiments in which barley plants were infiltrated with solutions of xylose, maltose, lactose, mannitol, sorbitol, glyceric aldehyde, and the sodium salts of gluconic and pyruvic acids. In these experiments the plants were depleted of sucrose in the usual manner before infiltration with the solutions. A sample of plants was also infiltrated with glucose in order to determine the rate of sucrose formation from the particular sugar with respect to glucose.

TABLE III

SUCROSE SYNTHESIS IN SUCROSE DEPLETED BARLEY SHOOTS AFTER INFILTRATION WITH 5 PER CENT. SOLUTIONS OF VARIOUS SUGARS OR RELATED SUGAR COMPOUNDS AND ALLOWED TO REMAIN FOR 20 HOURS AT 22° C.

SAMPLE	DESCRIPTION OF SAMPLE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	SUCROSE PER 5 GM. OF FRESH WEIGHT	RELATIVE RATE OF SUCROSE FORMATION
		%	%	%	*mg.*	
1	Initial—after depletion	1.23	1.43	0.19	9.5	.....
2	Depleted, then water infiltrated (20 hours)	0.79	0.90	0.10	5.0	.....
3	Glucose infiltrated	1.80	2.58	0.74	37.0	100.0
4	Xylose infiltrated	2.34	2.47	0.12	6.0	.....
5	Maltose infiltrated	2.09	2.90	0.77	38.5	105.0
6	Lactose infiltrated	2.03	2.60	0.54	27.0	68.8
7	Mannitol infiltrated	0.79	0.92	0.12	6.0	.....
8	Sorbitol infiltrated	0.60	0.67	0.07	3.5	.....
9	Glyceric aldehyde	1.00	1.40	0.38	19.0	43.8
10	Gluconic acid (Na salt)	1.20	1.37	0.16	8.0	.....
11	Pyruvic acid (Na salt)	0.70	0.88	0.17	8.5	.....

Sucrose was formed in the barley plants from maltose, lactose, and glyceric aldehyde. The rate of sucrose synthesis from these sugars with respect to glucose is 105 per cent., 68.8 per cent. and 43.8 per cent. respectively. It is of interest to note that the disaccharides, maltose and lactose, are used by the plants for the synthesis of sucrose and that maltose is utilized to about the same extent as glucose. Apparently the barley plant possesses enzymes which readily hydrolyze these disaccharides to their respective monosaccharides, which are then used for sucrose synthesis. Xylose, mannitol, sorbitol, and the salts of gluconic and pyruvic acids were not utilized. The utilization of a pentose sugar for sucrose synthesis would require an additional carbon atom for the preliminary formation of a hexose. Evidently the barley plant does not possess a mechanism for such a transformation. There are apparently also no enzymes which could reduce sugar acids or oxidize sugar alcohols to their corresponding aldohexoses. The formation of a considerable amount of sucrose from glyceric aldehyde shows that the plant is able to condense this tricarbon compound to glucose or fructose.

In table IV the results with etiolated plants are presented. The plants were germinated and grown in a culture solution in the dark for about 2 weeks; they were light yellow and absolutely devoid of chlorophyll.

TABLE IV

SUCROSE SYNTHESIS IN ETIOLATED BARLEY SHOOTS INFILTRATED WITH 5 PER CENT. SOLUTIONS OF VARIOUS SUGARS, AFTER RESPIRATION IN THE DARK FOR 24 HOURS, AND THEN ALLOWED TO REMAIN FOR 18 HOURS AT 22° C.

SAMPLE	DESCRIPTION OF SAMPLE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	SUCROSE PER 5 GM. OF FRESH WEIGHT
		%	%	%	*mg.*
1	Initial untreated	0.50	0.50	0.0	0.0
2	Depleted for 24 hours	0.42	0.42	0.0	0.0
3	Glucose infiltrated	1.30	1.83	0.50	25.0
4	Sucrose infiltrated	1.14	1.66	0.49	24.5
5	Maltose infiltrated	0.87	1.41	0.51	25.5
6	Galactose infiltrated	1.30	1.58	0.27	13.5

Sucrose formation takes place in barley plants which are devoid of chlorophyll. The etiolated plants did not contain any sucrose (sample no. 1). Sample no. 4, infiltrated with sucrose, did not raise the sucrose content above the one infiltrated with glucose; instead, it raised the value of reducing sugars. As shown in the table IV, the value of the reducing sugars in the blank sample after respiration for 24 hours was 0.42 per cent. After being infiltrated with glucose and allowed to remain for 18 hours, the respired barley plants contained 0.50 per cent sucrose and 1.30 per cent. glucose. The plants infiltrated with sucrose and allowed to remain for the same period of time, contained about the same amount of sucrose, 0.49 per cent., and 1.14 per cent. reducing sugars. It appears that an excess of reducing sugars induces synthesis of sucrose, while an excess of sucrose favors the reverse process, hydrolysis.

In order to show whether the synthesis of sucrose from a monosaccharide requires oxygen, the following experiment was carried out: barley samples infiltrated with 5 per cent. glucose were placed in 3 desiccators. The first contained air saturated with water vapor, in the second the air was replaced by oxygen, and in the third by nitrogen. The plants were allowed to remain in the dark for 24 hours. Originally they contained 47.5 mg. of sucrose per 5 gm. of fresh weight, and respired for 36 hours to a level of 19.5 mg. The results are shown in table V.

Sample no. 4, in the air, synthesized 58.5 mg. per 5 gm. of tissue. When the air was replaced by nitrogen only 22.5 mg. of sucrose per 5 gm. of tissue were synthesized, a value slightly higher than the blank, 19.5 mg. Sample no. 3, in pure oxygen synthesized 63.0 mg. of sucrose per 5 gm. of tissue, a

TABLE V

SUCROSE SYNTHESIS IN BARLEY SHOOTS INFILTRATED WITH 5 PER CENT. SOLUTIONS OF GLUCOSE AND EXPOSED TO AN ATMOSPHERE OF AIR, NITROGEN, AND OXYGEN FOR 24 HOURS AT 22° C.

SAMPLE	DESCRIPTION OF SAMPLES	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	SUCROSE PER 5 GM. OF FRESH WEIGHT
		%	%	%	*mg.*
1	Initial after depletion (in air)	1.37	1.78	0.39	19.5
2	Nitrogen	2.00	2.47	0.45	22.5
3	Oxygen	2.03	3.36	1.26	63.0
4	Air	2.10	3.33	1.17	58.5

value slightly higher than in air. This indicates that the synthesis of sucrose is an aerobic, or at least partly aerobic, process.

Invertase is generally considered to be specific for sucrose. The question of strict specificity of this enzyme is of prime importance in this investigation, since the conclusion that sucrose is synthesized from supplied monosaccharides rests entirely upon this assumption. There is a possibility that a compound other than sucrose might be formed which could be hydrolyzed by invertase. For example, when the plant is supplied with mannose or galactose, some non-reducing disaccharide might be formed which would also be subject to the attack of invertase. The release of free monosaccharides would produce a high reducing value similar to the hydrolysis of sucrose. If the reducing value, after invertase hydrolysis, is in reality due to sucrose (since sucrose contains a molecule of fructose) half of the increase of that value should be approximately equal to a corresponding increase in fructose. To verify this point, barley plants were infiltrated with 5 per cent. solutions of glucose, mannose, and galactose, and the increase in sucrose obtained by the invertase method was compared with the increase in fructose determined by ROE's method (**11**).

The results in table VI show that, after supplying galactose or mannose, the increase in the reducing value, by hydrolysis with invertase, corresponds approximately with the increase in fructose. This indicates that the carbohydrate synthesized is sucrose.

Discussion

The synthesis of sucrose in barley when either glucose, fructose, mannose, or galactose is supplied, indicates that there is a mechanism in the plant, which renders the conversion of these monosaccharides possible. HARTT (**4**) working with blades of sugar cane found that mannose was not utilized to form sucrose. The power of interconversion of the different monosaccharides apparently is dependent on the enzymic system and varies

TABLE VI

SUCROSE SNYTHESIS IN SUGAR DEPLETED BARLEY SHOOTS, ESTIMATED FROM INCREASE IN TOTAL REDUCING VALUE AND FRUCTOSE CONTENT AFTER HYDROLYSIS.

SAMPLE	DESCRIPTION OF SAMPLE	TIME INTERVAL AFTER INFILTRATION	REDUCING SUGARS	TOTAL SUGAR	SUCROSE	SUCROSE PER 5 GM. FRESH WEIGHT	FRUCTOSE PER 5 GM. FRESH WEIGHT	NET INCREASE OF FRUCTOSE PER 5 GM. FRESH WEIGHT
		hrs.	%	%	%	*mg.*	*mg.*	*mg.*
1	Initial—untreated	.....	0.43	0.77	0.32	16.0	10.8	.....
2	Depleted—36 hours	.....	0.37	0.37	0.0	0.0	6.0	.....
3	Galactose infiltrated	1	1.03	1.10	0.07	3.5	6.5	0.5
4	Galactose infiltrated	23	0.83	1.07	0.23	11.5	9.8	3.8
5	Galactose infiltrated	28	0.91	1.17	0.25	12.5	10.8	4.8
6	Mannose infiltrated	1	1.60	1.67	0.07	3.5	7.3	1.3
7	Mannose infiltrated	23	1.60	1.93	0.31	15.5	12.5	6.5
8	Mannose infiltrated	28	1.87	2.24	0.35	17.5	17.3	11.3
9	Glucose infiltrated	1	1.60	1.75	0.14	7.0	10.3	4.3
10	Glucose infiltrated	23	1.17	1.63	0.44	22.0	21.0	15.0
11	Glucose infiltrated	28	1.37	1.87	0.48	24.0	24.3	18.3

with different plants. The mutual interconversion of glucose, fructose, and mannose can be accomplished in vitro in very dilute alkaline solution through the LOBRY-BRUYN enolic transformation. Because of the ease with which glucose and fructose are interconverted in plants, it is considered possible that the transformation takes place through this mechanism. The fact that mannose was also found to be converted to glucose and fructose in the barley plant strengthens the view that the interconversion of these three monosaccharides in the plant takes place by the mechanism of enolization, but there is still no experimental evidence to support this assumption. It is even more difficult to account for the transformation of galactose into glucose, since this sugar has no common enolic form with glucose or fructose. The theoretically possible change of position of the fourth hydroxyl group in the glucose molecule by the mechanism of the WALDEN inversion, whereby it could be converted into galactose, also has no experimental proof.

The existence of hexosephosphates in plants shown by several investigators (**1, 7, 13**) in recent years strongly indicates that phosphorus plays an important rôle in the mechanism of sucrose formation. The isolation of a mixture of glucose and fructose-phosphoric acid esters from pea leaves by one of the writers (**7**) suggests that phosphorylation of the glucose and fructose components is probably a necessary step in the synthesis of sucrose in the plant. Support of this view can be found in the work of KURSANOV and KRYUKOVA (**9**). These authors showed that synthesis of sucrose in chicory and sugar beet leaves was always accompanied by an accumulation of phosphoric esters, also the synthesis of esters by sucrose synthesis. Under conditions of phosphate deficiency the capacity of the plant to synthesize sucrose decreased considerably. The introduction of phosphate in such plants restored their normal synthetic capacity. SYSSAKYAN (**12**) has also found that in the case of phosphorus-starved sugar beet leaves, the synthesis of sucrose was inhibited to a considerable extent as compared with normal plants. HANES's (**2**) recent important contribution, that of synthesizing starch from glucose-l-phosphate in the presence of phosphorylase from potato tubers, demonstrates the important rôle of phosphorus in the mechanism of carbohydrate synthesis. It is suggested that in the interconversion of monosaccharides some intermediate phosphorylated compound might also be involved.

Mannitol, sorbitol, and gluconic acid were not utilized by barley to form sucrose. Apparently the plant did not contain any enzymes which could oxidize the sugar alcohols or reduce the sugar acid. The formation of glucose from glyceric aldehyde indicates that sugars may be formed from tricarbon atom compounds containing a reducing group.

Summary

1. Sucrose can be synthesized in barley plants when either of the following monosaccharides are supplied: glucose, fructose, mannose, and galactose. It is therefore evident that the plant possesses a mechanism to convert these monosaccharides into glucose or fructose.

2. Maltose and lactose can also be utilized by barley for sucrose formation. The plants, apparently, possess enzymes which are able to hydrolyze these disaccharides to their respective monosaccharides, which are then used for the synthesis of sucrose.

3. Synthesis of sucrose from monosaccharides can take place in etiolated plants in the dark. The process is therefore independent of light and does not require chlorophyll.

4. Arabinose, xylose, mannitol, sorbitol, and gluconic and pyruvic acids were not utilized by the plant for sucrose formation.

5. When excised barley shoots respired for 24 hours the sucrose gradually diminished, apparently due to hydrolysis, but the reducing sugars remained approximately at a constant level throughout that period. It was only after the sucrose was entirely depleted, that a diminution in the reducing sugars could be observed. It was also shown that an excess of reducing sugars induces synthesis of sucrose, while an excess of sucrose favors the reverse process, hydrolysis.

6. Synthesis of sucrose did not occur without the presence of oxygen, which indicates that this process is aerobic.

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LITERATURE CITED

1. BURKARD, J., and NEUBERG, C. Zur Frage nach der Entstehung des Rohrzuckers. Biochem. Zeitschr. **270**: 229–234. 1934.
2. HANES, C. S. Enzymic synthesis of starch from glucose-l-phosphate. Nature **145**: 348–349. 1940.
3. HARTT, C. E. The synthesis of sucrose by excised blades of sugar cane. The Hawaiian Planters' Record. **41**: 33–46. 1937.
4. ————————. Report of enzyme laboratory. Proc. Hawaiian Sugar Planters' Association. Rept. committee in charge Exp. Sta. **58**: 111–116. 1938. (Pub. 1939).
5. HASSID, W. Z. Determination of reducing sugars and sucrose in plant materials. Ind. Eng. Chem. Anal. Ed. **8**: 138–140. 1936.

6. ————————. Determination of sugars in plants by oxidation with ferricyanide and ceric sulfate titration. Ind. Eng. Chem. Anal. Ed. **9**: 228–229. 1937.
7. ————————. Isolation of a hexosemonophosphate from pea leaves. Plant Physiol. **13**: 641–647. 1938.
8. KURSANOV, A. The use of the vacuum infiltration method for the determination of the synthetic and hydrolytic actions of invertase in living plant tissues. Biokhimiya **1**: 269–294. 1936.
9. ————————, AND KRYUKOVA, N. Participation of phosphatase in the synthesis of sucrose. Biokhimiya **4**: 229–239. 1939.
10. NELSON, J. M., and AUCHINCLOSS, R. The effects of glucose and fructose on the sucrose content in potato slices. Jour. Amer. Chem. Soc. **55**: 3769–3772. 1933.
11. ROE, J. H. A colorimetic method for the determination of fructose in blood and urine. Jour. Biol. Chem. **107**: 15–22. 1934.
12. SYSSAKYAN, N. M. The rôle of phosphates in the process of sugar accumulation in the sugar-beet. Biokhimiya. **1**: 301–320. 1936.
13. TÁNKÓ, B. Hexosephosphates produced by higher plants. Biochem. Jour. **30**: 692–700. 1936.
14. VIRTANEN, A. I., and NORDLUND, M. Synthesis of sucrose in plant tissue. Biochem. Jour. **28**: 1729–1732. 1934.

REFRACTIVE INDEX AS AN ESTIMATE OF QUALITY BETWEEN AND WITHIN MUSKMELON FRUITS[1,2]

T. M. Currence and Russell Larson

(with two figures)

Introduction

Many difficulties arise in connection with testing the eating quality of fruits. Table quality depends on taste to a great extent, and uniform agreement among different individuals as to the desirability of certain flavors seems to be an elusive objective. An evaluation may be obtained by getting numerous estimates in quantitative classes and averaging them. This, however, is laborious and generally too tedious for extensive use. The present work on muskmelons arose in connection with varietal improvement studies with this crop. It was evident that a heterozygous population produced progenies with wide differences in quality that were traceable to quality differences in their parents. Inconsistencies in such observations, however, indicated the desirability of more accurate determinations of quality than was possible by having a few individuals taste the fruits and give them quality ratings. Use of the hand refractometer that is commonly used in rapid testing of sugar beet juice for sugar content appeared to offer a solution to the problem, provided it gave a reliable index to eating quality. This method has been used by different workers (**1, 2**) for testing melon quality but there has not appeared in the literature any information as to the degree of error that may be involved when quality is estimated by this method. The data which follow are intended to show mainly the mathematical relation between such refractometer readings and quality ratings when the latter is determined by a number of organoleptic tests and to compare the variation that was found in such tests with the variation in estimates based on refractometer readings.

Materials and methods

In the initial study, juice of 30 muskmelons was tested by means of a Zeiss hand refractometer, a rapid and simple test for total soluble solids contained in the juice. Whereas the index of refraction of a substance is the ratio of the sine of the angle of incidence to the sine of the angle of refraction, the

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scale of the Zeiss hand refractometer is graduated from 0 to 30 in percentage of dry substance in the solution. The indices of refraction for 0, 5, 10, 15, 20, 25 and 30 per cent. by weight of aqueous sucrose solutions at 20° C. are 1.3330, 1.3403, 1.3479, 1.3557, 1.3639, 1.3723, and 1.3811 respectively.

The strains represented were progenies of a single heterozygous plant with varying amounts of inbreeding and selection covering one to five generations. The pedigree of the original plant is not known, but it is certain from types segregated that honeydew entered into the parentage. The samples of juice for refractometer tests were taken from the central area of the fruits and the fruits were then rated for quality by 19 people. Five arbitrary classes were set up ranging from one to five, with one being the lowest class for eating quality and five being the highest. Only whole numbers were used by the testers in rating the fruits. The readings obtained were averaged for each of the fruits and this mean was used as the quality rating of the respective melon.

A second set of observations, somewhat similar but differing in certain details, was collected. Ten fruits taken at random from a field of mixed strains were tested by the refractometer, juice being taken from the central area of the fruits. The fruits were quartered by cutting them longitudinally and transversely, the longitudinal cut being made as nearly as possible parallel to the surface of the ground upon which the melon rested as it developed. Figure 1 illustrates the four sections and the identifying numbers. It is obvious that a larger number of fruits would have been desirable but it was thought that 40 samples was near the maximum number that could be properly tested by an individual at one time.

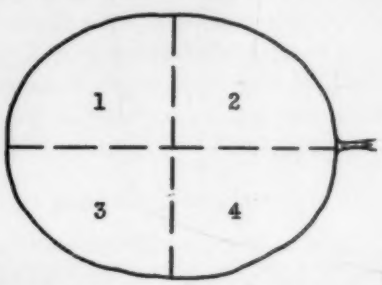

FIG. 1. The cuts made to divide muskmelon fruits into four sections and the respective numbers of the sections.

The 40 sections were each tested by the hand refractometer and laid out at random. Then eighteen persons tested, independently, each of the 40 samples and rated them for quality in classes ranging from one to five. This arrangement provides comparisons and tests of different parts of the fruits which were not possible in the original test.

Results

Data on quality from the initial test

The analysis of variance for the first test is summarized in table I. It is evident that fruits differed significantly in quality, and that the testers differed significantly in rating them. The F value exceeds the 1 per cent.

TABLE I

Analysis of variance of quality ratings on 30 muskmelon fruits

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	Standard error
Melons	29	223.0	7.70	*	.....
Testers	18	71.0	3.94	*	.....
Error (melons × testers)	522	452.3	0.866	.....	0.930
Total	569	746.3	.....	.....	.....

* Exceeds 1 per cent. point.

point in both instances. The standard error of 0.930 indicates the high degree of variability that occurs from such a test. The standard error of the mean of 18 tests is $\frac{0.930}{\sqrt{18}}$ or 0.219 which is taken as the standard error of the melon means. A difference of approximately 0.620 between means is statistically significant and one of 0.850 is highly so. Calculations show that ratings by 7 testers would have been needed to establish the statistical significance of a difference of one class between two melons. This emphasizes the relationship of taste variations to measurement of quality differences. The relationship is more emphatic if the calculation is made to find the number of tests needed to establish statistical significance of a difference of 0.5 of a class. It is found that the standard error of the mean must reduce to 0.176 which would require approximately 28 observations.

The refractometer readings as shown in table 6 cover a wide range and undoubtedly represent melons of several distinct classes. The relation of these results to the quality ratings will be discussed more fully under the heading of correlation.

Data on quality from the second test

The possibility that the blossom end of a muskmelon is higher in quality than the stem end is frequently encountered and likewise it is possible that the top and bottom halves of the fruit differ in quality. In order to test these possibilities, the analysis of variance was made as summarized in table II. Since this analysis shows the sections to be significantly different, it is desired to compare the means for differences. Figure 2 A is a diagrammatic presentation of the quality means of the four sections. A difference of

TABLE II

ANALYSIS OF VARIANCE FOR QUALITY SCORES OF FOUR SECTIONS OF EACH OF 10 MUSKMELON FRUITS BASED ON INDIVIDUAL ESTIMATES OF 18 TESTERS

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F	STANDARD ERROR
Testers	17	78.28	4.61	†	.....
Melons	9	375.73	41.75	†	.....
Sections	3	12.97	4.32	*	.....
Melons × sections error (A)	27	37.84	1.40	†	1.18
Testers × melons	153	240.00	1.57	†	.....
Error (B)	510	324.18	0.64	.....	0.80
Total	719	1069.00	.....	.....	.....

* Exceeds 5 per cent. point.
† Exceeds 1 per cent. point.

approximately 0.211 is significant and one of approximately 0.286 is highly significant. Therefore, although the difference is small, section 2 differs significantly from the other three sections but none of these differ among themselves by statistically significant amounts. It is apparent that quality ratings differed between halves of the fruits. The blossom end was slightly but significantly higher than the stem end, and the bottom half was insignificantly higher than the top half.

The means of the halves have a standard error of 0.062. These are also

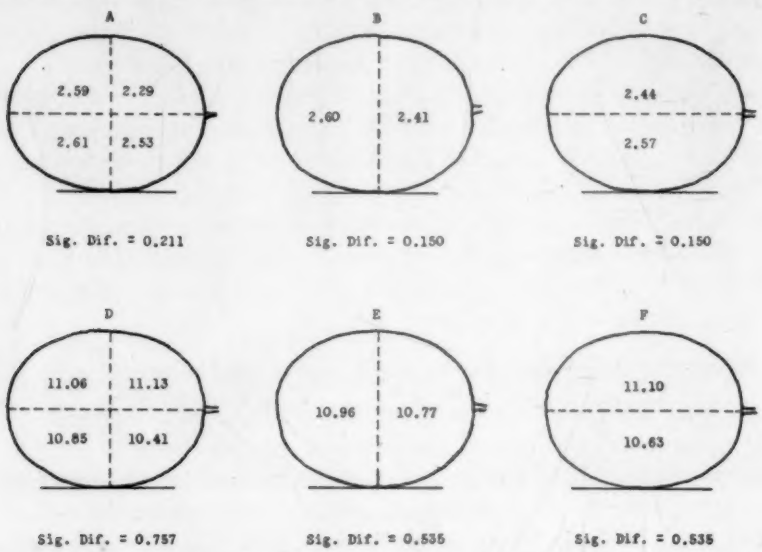

FIG. 2. Diagrammatic presentation of quality ratings A, B, and C and refractometer readings D, E, and F of different areas of muskmelon fruits.

shown diagrammatically in figures 2 B and 2 C. A difference of 0.150 between these is statistically significant and one of 0.202 is highly so. It is apparent that the two ends of the fruit show a difference that is significant. In the case of the upper and lower halves, the difference is smaller and does not reach the five per cent. point. The odds, however, are approximately 12:1 against a difference of this size being the result of random samples. The data definitely seem to suggest minor differences in quality between various parts of the fruits and therefore the desirability of defining areas when the fruits are tested for quality.

The interaction sums of squares, shown in table II, are of some interest. That of testers × melons, being significant, indicates that various testers rated the same melons differently. Stated otherwise, the quality of a melon may appeal to a certain tester but would rate low in the opinion of another. Thereby the point is illustrated that individual estimates vary as to what constitutes desirable or undesirable quality. The detailed results on this interaction are shown in table III. The differences in estimates may be readily seen by observing a typical case such as the ratings given melons A and I by testers 11 and 13.

TABLE III

THE QUALITY RATINGS OF 10 MUSKMELON FRUITS AS DETERMINED BY 18 TESTERS RATING FOUR SAMPLES OF EACH FRUIT

Tester	Melon										Mean
	A	B	C	D	E	F	G	H	I	J	
1	3.50	2.00	4.25	2.00	1.00	3.00	2.00	3.00	1.75	2.75	2.52
2	4.25	2.50	5.00	1.75	1.00	4.00	2.25	2.75	3.00	1.25	2.77
3	3.00	2.25	4.75	2.00	1.00	3.75	3.00	2.75	2.75	3.75	2.90
4	3.00	1.00	3.50	2.75	1.00	2.50	3.00	1.50	2.75	1.75	2.27
5	2.00	1.25	2.00	1.75	1.50	2.00	2.25	2.50	2.50	1.00	1.87
6	1.75	3.00	5.00	2.00	1.25	2.50	2.50	1.50	3.75	1.50	2.47
7	2.50	2.00	3.00	3.00	1.00	2.75	1.75	2.00	3.75	1.25	2.30
8	3.50	2.25	3.25	2.75	1.00	3.00	2.75	2.75	4.50	3.25	2.90
9	3.50	3.00	4.50	2.50	1.25	3.00	2.75	2.00	3.75	3.00	2.92
10	3.50	2.00	4.50	3.00	1.00	3.25	2.25	2.25	3.75	2.75	2.82
11	3.75	1.00	4.00	2.00	1.50	3.50	1.75	2.00	2.75	1.50	2.37
12	2.75	2.75	5.00	3.75	1.25	3.00	3.00	2.50	3.75	3.50	3.12
13	1.25	1.75	4.75	2.50	1.00	2.50	2.75	2.75	4.25	1.25	2.47
14	3.75	1.75	4.50	1.75	1.00	2.75	1.75	2.50	3.25	1.50	2.45
15	2.50	2.75	2.00	3.50	1.00	3.75	2.25	2.00	3.25	1.25	2.42
16	2.75	1.75	3.25	2.00	1.00	2.25	2.50	1.50	3.50	1.50	2.20
17	1.75	1.25	3.25	2.50	1.25	2.00	2.25	2.50	3.50	1.00	2.12
18	1.75	2.50	2.25	2.75	1.00	2.50	2.25	1.50	3.50	1.50	2.15
Mean	2.82	2.04	3.82	2.46	1.11	2.89	2.39	2.24	3.33	1.96	.....

The melons × sections interaction was significantly greater than that for error (B). Table IV shows the data on this interaction. It is evident that considerable variation occurred in a certain section for different melons.

TABLE IV

QUALITY RATINGS FOR FOUR SECTIONS OF EACH OF 10 MUSKMELON FRUITS AS DETERMINED BY 18 TESTERS PER SAMPLE

MELON	SECTION OF MELON				MEAN
	1	2	3	4	
A	2.78	2.89	2.67	2.94	2.82
B	2.17	1.78	2.39	1.83	2.04
C	4.11	3.33	4.33	3.50	3.82
D	2.11	2.39	2.44	2.89	2.46
E	1.22	1.00	1.05	1.17	1.11
F	3.06	2.67	3.06	2.78	2.89
G	2.72	2.00	2.17	2.67	2.39
H	2.39	1.94	2.22	2.39	2.23
I	2.94	3.06	3.83	3.50	3.33
J	2.44	1.78	1.94	1.67	1.96
Mean	2.59	2.28	2.61	2.53	.....

The interaction can be visualized by noting that section 1 had the highest rating in three of the fruits, section 2 in none of them, section 3 in three, and section 4 in two fruits. In two cases there was an equal rating between sections for the highest score; one and three in the case of fruit F, and one and four for fruit H. Thus, it is seen that although section 1 had the high score in most cases, section 3 had the highest general average, and that the section ratings differed in different melons.

CONSISTENCY OF EXPERIENCED TESTERS

Considering the variation in estimates by the organoleptic tests the question may arise as to the possibility of reducing this if the individuals making the ratings have had previous experience at it. It may be possible that extensive sampling would enable an individual to be more consistent in his ratings. Three of the samplers in the second test were individuals who had had considerable experience at testing various fruits and vegetables in research work. Attempting to determine the effect of this background on their comparative ratings, correlation coefficients were calculated between the mean melon ratings of each of these three and the mean of each melon for 12 of the other testers. The scores of the three remaining testers were also correlated with the scores of the 12 combined and were found to give essentially the same correlations as that for the experienced testers. It appears in this instance, that the experienced testers were not superior over three testers selected at random in estimating the mean of several tastes.

REFRACTOMETER DATA FROM THE SECOND TEST

When the 40 refractometer readings are divided and analyzed, as shown in table V, it is seen that statistical significance between sections is not indi-

cated although the quality differences shown by figures 2 A, 2 B, and 2 C, are significant. This suggests that the quality score differences for different sec-

TABLE V

ANALYSIS OF VARIANCE FOR 40 REFRACTOMETER READINGS ON FOUR SECTIONS OF EACH OF 10 MELONS

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F	STANDARD ERROR
Melons	9	217.25	24.14	*	.....
Sections	3	4.24	1.41	.....	.....
Error	27	18.51	0.68	.....	0.825
Total	39	240.78	.....	.....	.....

* Exceeds 1 per cent. point.

tions were possibly the result of factors other than soluble solids, as indicated by the refractometer readings. TUCKER (**4**) has shown that the sugar content varies in different parts of a watermelon fruit. SCOTT and MACGILLIVRAY (**3**) obtained uniform readings in soluble solids for longitudinal sections of muskmelons but found an increasing gradient from the stem to the blossom end for cross sections. In the present study the odds for some of the comparisons, although not 20 : 1, are rather high. As example, section 3 compared with section 4 gives odds of 7 : 1; and the top half compared with the bottom half gives odds of approximately 15 : 1. It is of interest that these differences are not in the same relationship as the differences between the quality scores. This seems to be further evidence that the quality differences between sections may result from factors other than sugar content. From the above it is understandable that the analysis of covariance failed to show significant correlation between quality score and refractometer readings within fruits although it was quite definite between fruits.

CORRELATION DATA

The correlation coefficient for the first set of data calculated between the refractometer readings and the quality score means shown in table VI is + 0.636 and is highly significant. The regression of quality on refractometer reading is 0.167. This is the average amount by which the quality score varied with a unit change in refractometer reading. Using this regression coefficient and a refractometer reading, it is possible to estimate the quality score. The accuracy of the estimate is indicated by the last column in table VI. The standard error of estimate is 0.493, which may be considered as an average of the differences between observed and estimated quality. This is a decidedly lower value than the standard error of 0.930 shown in table I.

In the second test, having the correlation broken up, it is possible to consider the relationship for within melons and between melons by Fisher's

TABLE VI

REGRESSION OF QUALITY ON REFRACTOMETER READING FOR MUSKMELONS (QUALITY RATING IS THE MEAN AS DETERMINED BY THE ESTIMATES OF 19 INDIVIDUALS GIVING RATINGS OF 1 TO 5 FOR QUALITY)

FRUIT NO.	REFRACTOMETER READINGS	OBSERVED QUALITY	ESTIMATED QUALITY	OBSERVED – ESTIMATED
1	12.0	2.63	3.03	−0.40
2	11.8	2.37	3.00	−0.63
3	9.5	1.95	2.60	−0.65
4	12.8	2.47	3.17	−0.70
5	8.5	1.58	2.45	−0.87
6	11.0	2.79	2.87	−0.08
7	13.4	3.68	3.27	0.41
8	11.2	2.89	2.89	0.00
9	12.0	3.10	3.03	0.07
10	8.0	2.63	2.36	0.27
11	9.2	3.42	2.56	0.86
12	12.5	3.05	3.12	−0.07
13	13.0	3.26	3.20	0.06
14	7.6	2.74	2.30	0.44
15	8.2	2.11	2.40	−0.29
16	9.2	3.37	2.56	0.81
17	8.4	3.26	2.33	−0.93
18	13.4	2.95	3.27	−0.32
19	10.0	2.89	2.70	0.19
20	6.8	1.79	2.16	−0.37
21	9.4	2.58	2.60	−0.02
22	8.0	2.11	2.36	−0.25
23	14.0	3.37	3.36	0.01
24	9.4	3.05	2.60	0.45
25	12.2	3.16	3.07	0.09
26	9.4	2.58	2.60	−0.02
27	6.2	2.00	2.06	−0.06
28	5.0	1.37	1.86	−0.49
29	7.0	1.95	2.20	−0.25
30	10.8	3.89	2.83	0.96

analysis of covariance method. A regression coefficient for between melons is arrived at as well as one for the association within melons or between sections. As previously stated, however, it was found that the correlation within fruits was not significant, and the correlation between refractometer reading and quality score mean for between fruits was essentially the same as that for total. Furthermore, the one refractometer reading originally taken on the 10 fruits when correlated with the quality means gave approximately the same value as that when the mean of four refractometer readings was used.

Inasmuch as this single reading approximates the results of the more extensive readings and represents considerable saving in time, it seems desirable to limit further analysis to the one reading data. The correlation coefficient between this value and quality score is +0.862 and is highly significant. The regression of quality on refractometer reading is 0.264 in this instance. The weight in grams of the 10 melons also was correlated with

quality, the value being + 0.655 and slightly above the five per cent. point. This is surprising and the relationship probably is not generally true of muskmelon fruits. Fruit size being a character easily measured, however, it should be more adequately studied in relation to quality between types as well as within uniform strains. The multiple correlation coefficient between the three measurements was found to be + 0.909. As shown by table VII, the standard error of estimate is 0.342. Based on this 10-melon sample, it is apparent that estimating quality from the multiple regression is relatively accurate.

TABLE VII

REGRESSION OF QUALITY SCORE ON FRUIT SIZE AND REFRACTOMETER READINGS FOR MUSKMELON FRUITS (QUALITY RATINGS DETERMINED BY 18 INDIVIDUALS MAKING FOUR TESTS PER FRUIT)

FRUIT IDENTIFICATION	WEIGHT IN GRAMS	REFRACTOMETER READING	OBSERVED QUALITY RATING	ESTIMATED QUALITY	DIFFERENCE
A	1490	10.4	2.81	2.22	0.59
B	1350	11.0	2.04	2.33	−0.29
C	3850	13.3	3.82	3.69	0.13
D	1290	12.2	2.45	2.63	−0.18
E	970	6.0	1.11	.90	0.21
F	2000	12.6	2.88	2.95	−0.07
G	2640	11.0	2.38	2.72	−0.34
H	1550	9.6	2.23	2.02	0.21
I	1380	13.8	3.33	3.08	0.25
J	1600	10.9	1.95	2.38	−0.43

Comparing the respective standard errors, it is noted that the above 0.342 is approximately equal to that of 12 testers sampling by tasting, since 1.18 divided by the square root of 11.9 approximately equals 0.342. Thus, it seems that the simple weight and refractometer tests were useful tools for calculating eating quality under the conditions of this test. The calculation for getting the estimated quality is 0.0003 times the weight in grams plus 0.264 times the refractometer reading and this sum minus 0.975. Although the calculation is a simple one, a melon breeder would seldom use it in testing selections. The simple procedure of comparing refractometer readings would suffice in most instances.

Summary and conclusions

Thirty muskmelons representing a mixture of types were tested for the percentage of soluble solids in the juice by means of a hand refractometer. The melons were then rated quantitatively in ascending classes from one to five by 19 people tasting them. Analysis of the data indicates that the melons were significantly variable in quality and that testers varied significantly, some tending to rate the fruits low and others to rate them high. The standard error of 0.930 illustrates the difficulty of satisfactorily

classifying the fruits by tasting unless the mean of a number of opinions is obtained. Ratings by three experienced testers did not approach the mean scores appreciably more closely than the ratings of three testers selected at random.

In a second test, 10 muskmelon fruits were cut into four sections after they were tested by the refractometer. Each section was tested separately by the refractometer and also rated for quality by 18 testers. The results indicate slight but significant differences in quality between different parts of the fruits. The blossom end averaged higher than the stem end. The bottom half was not significantly higher than the upper half. Interaction data show poor agreement among the different individuals in rating the fruits for quality. Also it is shown that certain sections were high in some melons and low in others. Analysis of the refractometer readings do not show statistically significant differences between the different parts of the fruits and such differences as did occur were somewhat opposed to the differences in quality. It is, therefore, suggested that the quality differences between sections may have resulted from factors other than those measured by the refractometer.

For the first set of data, quality rating estimates based on the regression of quality on refractometer readings gave an error of estimate appreciably lower than the standard error by tasting. A correlation coefficient of + 0.636 and a regression coefficient of 0.167 were found. In the second lot of data, there was positive correlation between fruit weight and quality score (+ 0.655) as well as between refractometer reading and quality score (+ 0.862). Using multiple regression the error of estimate was further reduced so that it approximately equals the standard error of the mean of 12 samplers. The coefficient of correlation for the three characters is + 0.909. The information on the relation between fruit size and quality is thought to be incomplete but the extent to which refractometer readings can be used in estimating muskmelon quality is considered well demonstrated by the foregoing material.

THE MINNESOTA AGRICULTURAL EXPERIMENT STATION

LITERATURE CITED

1. PORTER, D. R., and BISSON, C. S. Total soluble solids and sugars in watermelons. Proc. Am. Soc. Hort. Sci. **32**: 596–599. 1934.
2. ————————, ————————, and ALLINGER, H. W. Factors affecting the total soluble solids, reducing sugars and sucrose in watermelons. Hilgardia **13**: 31–66. 1940.
3. SCOTT, G. W., and MACGILLIVRAY, JOHN H. Variation in solids of the juice from different regions in melon fruits. Hilgardia **13**: 69–79. 1940.
4. TUCKER, LOWELL R. Soluble solids in the watermelon. Plant Physiol. **9**: 181–182. 1934.

SOME EFFECTS OF SUPPLEMENTARY ILLUMINATION WITH MAZDA LAMPS ON THE CARBOHYDRATE AND THE NITROGEN METABOLISM OF THE ASTER (*CALLISTEPHUS CHINENSIS* VAR. HEART OF FRANCE)

RAYMOND WENGER

(WITH TWO FIGURES)

Introduction

Greenhouse crops show marked responses to supplementary artificial lighting with Mazda lamps (**4**). The present study was undertaken to determine whether there are differences in composition between groups of asters subjected to various supplementary light intensities and those which did not receive supplementary light; and to further ascertain if these differences in chemical composition, especially carbohydrates and nitrogenous compounds, can be correlated with the responses of the aster. This plant was chosen because it blooms in a long day and is suitable for study in short day.

GARNER and ALLARD (**3**) found that many of the plants with which they worked might be classified according to the length of day which favors their reproductive processes, namely: (a) short day type, (b) long day type, and (c) indeterminate type. MURNEEK (**7**), and PARKER and BORTHWICK (**10**) have shown that flower primordia can be initiated in Biloxi soybeans by subjecting the seedlings to a short day of 7 to 8 hours. After this induction period, the plants grown in a short photoperiod fruited while those grown in a long photoperiod remained vegetative. MURNEEK (**7**) points out that whether plants are vegetative or reproductive is apparently brought about directly by the length of day. PARKER and BORTHWICK state that changes in chemical composition that can stand in causal relationship to the initiation of flower primordia must occur before initiation can take place.

The work of KRAUS and KRAYBILL (**6**) has shown that the type of response in the tomato plant is associated with the carbohydrate content in relation to the available nitrogen supply. NIGHTINGALE (**8**) found that tomatoes grown under short day conditions and with available nitrogen supply produced vegetative growth. Long days with available nitrogen produced vigorously fruiting plants. In a later study (**9**) it was shown that Salvia, a short day plant, can be thrown into the vegetative state by lengthening the day. Carbohydrates and nitrates accumulate under short day conditions. When the plants are transferred to long day conditions, there is a loss of carbohydrates, and associated with this loss there is a more

rapid assimilation of nitrate and increased vegetative growth. MURNEEK (7) has indicated that as a result of a short photoperiod, seedling soybeans show a rapid accumulation of the storage forms of carbohydrates, especially starch, and a rapid synthesis of nitrogenous substances; these changes, however, probably have nothing to do with initiation of flower buds but rather promote development of the reproductive organs. ECKERSON (2) pointed out that reductase decreases in soybean plants which receive only 8 hours of daylight as compared with those which receive full daylight. It was thought that this might explain the accumulation of nitrates in a short day. HIBBARD (5), however, has found that the reductase activity was only partially inhibited in Biloxi soybeans by a short day, but showed no definite change as the plants responded to the different daylengths.

Methods

Seedlings, 1 to 1.5 inches, were transplanted to 2.5-inch clay pots and allowed to grow about one month before they were transferred to the different plots. The plants were transplanted to larger pots as needed.

In the first, or fall, series of experiments, four long day conditions and one short day condition were studied. All plants received normal daylight. The plants under long day conditions received supplementary illumination from Mazda lamps from 9 : 00 P.M. to 7 : 00 A.M. for a period of 10 hours each night; the plants under short day conditions, or controls, received only the normal daylight.

Representative plants were taken at each sampling date to have a minimum of 100 grams for the nitrogen determinations, 50 grams for the carbohydrate determinations, and 15 grams for dry weight studies. The leaves were clipped into small pieces and used for samples.

After the dry weights were obtained these same samples were used for total nitrogen (11). Another sample was thoroughly ground and a water extract made which served for the determination of the total water extractable nitrogen. After aliquots were drawn for the total water extractable nitrogen, the water extractable protein was coagulated by means of acetic acid and heat and filtered off. Aliquots were taken from the filtrate for the water soluble nitrogen. Additional aliquots served for the ammonia and nitrate (13), and amino nitrogen determinations (14). Total protein was obtained by subtracting the water soluble nitrogen from the total nitrogen.

In testing for carbohydrates, the samples were plunged into sufficient 95 per cent. hot ethyl alcohol to bring the alcohol content to 80 per cent; to this had been added 0.5 gram of $CaCO_3$. The alcohol extract was used for determining reducing substances and sucrose (1, 12). Starch was determined on the residue by the taka-diastase and subsequent hydrochloric acid hydrolysis method. The reducing power of the solution was determined as dextrose. These values were converted to starch by multiplying by 0.9.

Results

Experiment I

In a preliminary study[1] of the effect of supplementary light on asters observations were made on fresh weights, stem length, and time of flowering. Chemical analyses were also made on these plants. This work was repeated in more detail in experiments II and III.

Experiment II. first series, 1933

First sampling.—Aster seed was sown in flats August 26, 1933, transplanted to pots September 14, and transferred to supplementary light plots on September 24. The average greenhouse temperature was 55° F. The first sampling was taken on October 24, after 30 days of growth. Results of the analyses are given in table I.

TABLE I

Effect of supplementary illumination on the carbohydrate and nitrogen content of Aster. Expressed as percentage of the fresh weight. October 24, 1933. 30 days

Analyses	Foot candles				
	100	10	1	0.3	Control*
Averages fresh weight per plant in grams	4.17	4.25	3.99	3.58	2.81
Dry matter	12.62	12.35	13.28	13.75	16.24
Reducing substance	0.69	0.65	0.62	0.63	0.70
Sucrose	0.27	0.31	0.33	0.41	0.50
Starch	2.93	2.90	2.81	3.36	4.48
Total carbohydrates†	4.23	4.21	4.23	4.62	6.20
Total nitrogen	0.43	0.47	0.44	0.47	0.60
Soluble nitrogen	0.10	0.10	0.10	0.12	0.21
Protein nitrogen	0.33	0.37	0.34	0.35	0.39
Nitrate nitrogen	0.04	0.05	0.05	0.06	0.08
Amino nitrogen	0.03	0.03	0.04	0.04	0.06

* No supplementary light at night.

† Total carbohydrates are the sum of reducing substances and starch as dextrose, and sucrose as invert sugar.

Second sampling.—This sampling was taken on November 20, after 57 days of growth. The control plants were deep green in color and were low, spreading in appearance. Those plants which received supplementary light were a lighter green. Stems and leaves were analyzed separately. The results of these analyses are given in table II. The reaction of these plants to various degrees of supplementary lighting is shown in figure 1.

As shown in table II there is a progressive increase in fresh weight per plant as the supplementary light intensity increases. These data also show

[1] This was part of a cooperative project of the Horticultural and Agricultural Chemistry Departments, Purdue University Agricultural Experiment Station.

TABLE II

EFFECT OF SUPPLEMENTARY ILLUMINATION ON THE CARBOHYDRATE AND NITROGEN CONTENT OF ASTERS. EXPRESSED IN PERCENTAGE OF THE FRESH WEIGHT. NOVEMBER 20, 1935. 57 DAYS

ANALYSES	FOOT CANDLES				
	100	10	1	0.3	CONTROL*
Averages fresh weight per plant in grams	9.37	8.89	8.22	8.06	3.75
Dry matter	12.2	14.5	18.2	20.0	21.0
Reducing substance	1.34	1.51	1.48	1.44	1.30
Sucrose	0.32	0.42	0.51	0.70	0.71
Starch	1.87	2.44	2.42	2.00	1.90
Total carbohydrates†	3.77	4.68	4.71	4.40	4.16
Total nitrogen	0.31	0.30	0.33	0.30	0.49
Soluble nitrogen	0.05	0.04	0.04	0.06	0.07
Protein nitrogen	0.26	0.26	0.29	0.24	0.42
Nitrate nitrogen	0.03	0.02	0.02	0.03	0.04
Amino nitrogen	0.03	0.03	0.04	0.04	0.06

* No supplementary light at night.

† Total carbohydrates are the sum of reducing substances and starch as dextrose, and sucrose as invert sugar.

an increase in the percentage of total carbohydrates and starch from the control plants of the 10-foot-candle plot. The 100-foot-candle plot shows a decrease in total carbohydrates. The soluble nitrogen, nitrate, and amino nitrogen fractions are low in the lighted plots and high in the control plots.

This appears to indicate that in the control plants the nitrogen fractions are not utilized as rapidly or as efficiently in the formation of proteins and in vegetative extension as they are in the lighted plots.

FOURTH SAMPLING.—This was taken on Jaunary 30, 1934, after a growth

FIG. 1. The effect of supplementary light on the growth of Aster. First series, November 20, 1935. 57 days. (1), 100 foot candles; (2), 10 foot candles; (3), 1 foot candles; (4), 0.3 foot candles; (5), Control.

Plants were taken from plots which received 10 hours additional light each night of the intensities indicated.

period of 128 days. All plots were in bloom but not all of the plants in each plot. The controls were deeper green while the plants that received supple-

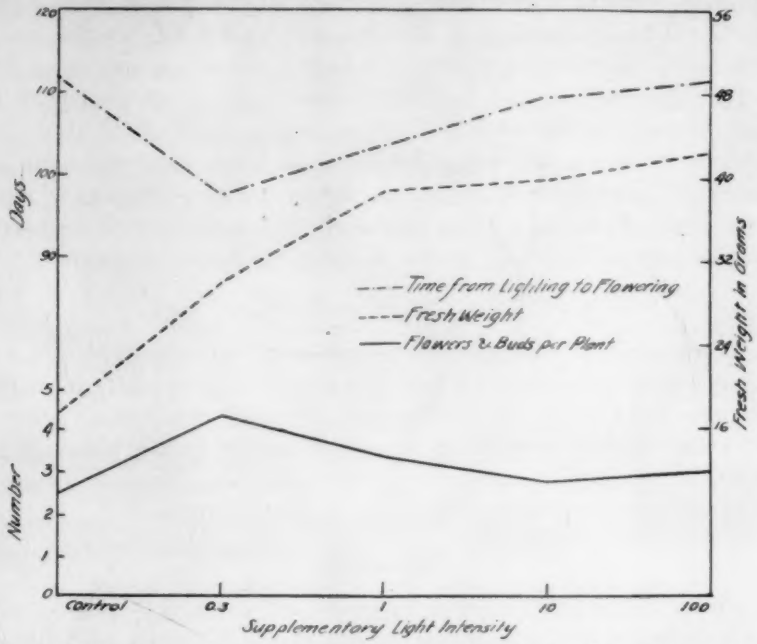

FIG. 2. The effect of supplementary light on time of flowering, fresh weight, and flowers per plant at time of harvest. First series, January 30, 1934. 128 days.

TABLE III

EFFECT OF SUPPLEMENTARY ILLUMINATION ON THE CARBOHYDRATE AND NITROGEN CONTENT OF ASTER. EXPRESSED IN PERCENTAGE OF THE FRESH WEIGHT. JANUARY 30, 1934. 128 DAYS

ANALYSES	FOOT CANDLES				
	100	10	1	0.3	CONTROL*
Average fresh weight per plant in grams	42.6	39.1	39.3	30.0	16.8
Dry matter	15.58	15.76	15.72	15.70	16.84
Reducing substance	0.90	0.88	0.93	1.18	1.27
Sucrose	0.32	0.38	0.38	0.40	0.35
Starch	2.41	2.52	2.27	1.89	2.02
Total carbohydrates†	3.92	4.03	3.87	3.71	3.90
Total nitrogen	0.22	0.20	0.21	0.24	0.39
Soluble nitrogen	0.10	0.07	0.07	0.08	0.15
Protein nitrogen	0.12	0.13	0.14	0.16	0.24
Nitrate nitrogen	0.008	0.01	0.01	0.01	0.01
Amino nitrogen	0.02	0.02	0.02	0.02	0.02

* No supplementary light at night.

† Total carbohydrates are the sum of reducing substances and starch as dextrose, and sucrose as invert sugar.

mentary light were a light green. Data relative to the time of flowering, fresh weights, and flowers per plant are presented in figure 2. Results of analyses are given in table III.

All of the buds were open on the plants in the 0.3-foot-candle plot while the plants in the 100-foot-candle plot had the most unopened buds; the plants in the control plot had the lowest number of unopened buds. Earliest flowering occurred in the 0.3-foot-candle plot.

Differences in composition expressed as percentages of the total carbohydrates and total nitrogen are not as evident in this cutting as in the first two cuttings (tables I, II). The differences in composition of the plants of the various plots diminish as the plants reach the flowering stage.

Experiment III. Second series, 1934

A second series of experiments was begun in February 1934. Only two groups received supplementary light (10 and 0.3 foot candles) for a period of 10 hours each night. A third group (control) received no additional light. Four samples were taken at approximately 30-day intervals. The fresh weight, height of plants, time of flowering, and chemical composition followed the same general trend as those in experiment II.

Discussion

The heights and fresh weights of the lighted (long day) plants increase as the supplementary light intensity increases. Withrow and Benedict (**15**) have shown that asters which receive 0.3 foot candle supplementary light or more have a greater dry weight than the control plants.

Carbohydrates.—As seen in table I the plants show a decrease in the percentage of total carbohydrates, sucrose, and starch as the supplementary light intensity increases. Associated with this decrease in percentage of total carbohydrates there is an increase in fresh and dry weights as compared with the controls. Though carbohydrates are produced in large quantities in the longer day of the lighted plants, they were utilized rapidly for growth and were stored but slowly in these plants. There was an accumulation of carbohydrates in the control plants. The percentage of total carbohydrates is relatively high in the plants of the 0.3-foot-candle plot (table I). It is probable that the removal of the products of photosynthesis was not as rapid as in the plants receiving the higher light intensities. Withrow (**15**) has suggested that the greater dry weight of plants receiving 0.3 foot candle, as compared with controls, may be due to an indirect effect of the supplementary light at night on the photosynthetic activity during the following day, possibly through a faster removal during the night of soluble carbohydrates accumulated during the day.

Nitrogen.—The control plants are higher in the percentage of nitrates

and water-soluble nitrogen in the early stages of growth as compared with the lighted plants. Associated with the lower percentage of nitrate and water-soluble nitrogen in the plants which receive supplementary light there is an increase of fresh weight and vegetative extension.

Summary

1. Asters receiving no supplementary illumination are least vegetative, bloom latest, and have the smallest number of flowers per plant. The plants are high in percentage of carbohydrate and soluble nitrogen but, in the short day length, are unable to utilize these compounds in vegetative extension.

2. Lengthening the day with Mazda lamps results in greater vegetative activity, larger number of flowers per plant, and earlier flowering.

3. Earliest flowering and largest number of flowers per plant occurs with the plants receiving supplementary illumination of the lowest intensity —0.3 foot candle. Such plants are medium in vegetative activity and relatively high in the percentage of carbohydrates and soluble nitrogen during the early stages of growth.

4. As the supplementary light intensity is increased to 100 foot candles vegetative activity increases, and the percentage of carbohydrates and soluble nitrogen decreases in the early stages of growth.

5. Significant differences in composition of the groups of plants occurred only in the early samplings. When the plants reach the flowering stage, the composition is very similar regardless of the treatment. Analyses of plants made at the time of flowering or maturity are of little value in interpreting carbohydrate-nitrogen relationships in growth and reproduction.

The writer is indebted to DR. H. R. KRAYBILL for the advice and encouragement given in carrying on this investigation. He also wishes to thank Mr. ROBERT B. WITHROW of the Horticultural Department, Purdue University Agricultural Station, for the installation and permission to use the lighting equipment.

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LITERATURE CITED

1. BERTRAND, GABRIEL. Le dosage des sucres reducteurs. Bull. Soc. Chim. Paris **35**: 1285. 1906.
2. ECKERSON, SOPHIA H. Conditions affecting nitrate reduction by plants. Contrib. Boyce Thompson Inst. **4**: 119–130. 1932.
3. GARNER, W. W., and ALLARD, H. A. Effect of the relative length of day and night and other functions of the environment on growth and reproduction in plants. Jour. Agr. Res. **18**: 553–606. 1920.

4. GREENE, LAURENZ, WITHROW, R. B., and RICHMAN, M. W. The response of greenhouse crops to electric light supplementing daylight. Indiana Agr. Exp. Sta. Bull. 366. 1932.
5. HIBBARD, AUDREY D. Photoperiodism and enzyme activity in the soybean plant. Missouri Agr. Exp. Sta. Res. Bull. 271. 1937.
6. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
7. MURNEEK, A. E. Biochemical studies of photoperiodism in plants. Missouri Agr. Exp. Sta. Res. Bull. 268. 1937.
8. NIGHTINGALE, G. T. Light in relation to the growth and chemical composition of some horticultural plants. Proc. Amer. Soc. Hort. Sci. **19**: 18–29. 1922.
9. ————. The chemical composition of plants in relation to photoperiodic changes. Wisconsin Agr. Exp. Sta. Res. Bull. 74. 1927.
10. PARKER, M. W., and BORTHWICK, H. A. Effect of photoperiod on development and metabolism of the Biloxi soy bean. Bot. Gaz. **100**: 651–689. 1939.
11. PUCHER, GEORGE, LEAVENWORTH, C. S., and VICKERY, H. B. Determination of total nitrogen of plant extracts in presence of nitrates. Ind. Eng. Chem. Anal. Ed. **2**: 191–193. 1930.
12. QUISUMBING, F. A., and THOMAS, A. W. Conditions affecting the quantitative determination of reducing sugars by Fehling solution. Elimination of certain errors involved current in methods. Jour. Amer. Chem. Soc. **43**: 1503–1526. 1921.
13. SESSIONS, A. C., and SHIVE, J. W. A method for the determination of inorganic nitrogen in plant extracts. Plant Physiol. **3**: 499–511. 1928.
14. VAN SLYKE, D. D. The quantitative determination of aliphatic amino groups. Jour. Biol. Chem. **12**: 277–284. 1912.
15. WITHROW, R. B., and BENEDICT, H. M. Photoperiodic responses of certain greenhouse annuals as influenced by intensity and wave-length of artificial light used to lengthen the daylight period. Plant Physiol. **11**: 225–249. 1936.

AN INEXPENSIVE INTEGRATING LIGHT RECORDER[1]

V. G. Sprague and E. M. Williams

(with three figures)

In recent years environmental factors, such as temperature and light, have been receiving increased consideration in experimental procedures. Under greenhouse conditions, the temperature and length of day can be controlled but it is impossible, except by excluding sunlight and supplying artificial light, to control the radiant energy which plants receive during a given growth period. The variation in the incident light during an experiment in the greenhouse or in the field may explain in part the differences between replications of the same experiment conducted at different times. Although it may not be possible to control the incident light available to experimental plants at various times, interpretations of the results obtained might be facilitated if the total amount of light which the plant had received were known. The length of day can be easily obtained from available data for each latitude but daily measurements of the total incident light are rarely obtainable. Instruments which will record the intensity of the natural light from day to day are available, but in order to obtain the total incident light, the area enclosed by the intensity curve must be determined. This is a tedious and time-consuming task if an experiment covers more than a few days, and becomes more difficult and less accurate on days when clouds are intermittent. To overcome the difficulties encountered with such a recording instrument, as well as to reduce the cost of equipment, an integrating light recorder was constructed from a few electrical parts obtainable from a radio dealer.

The principle of operation used in this instrument has been described by Anderson (**1**) who placed a small condenser in series with a phototube and a battery. A neon tube in parallel with the condenser flashed when the voltage across the condenser rose to the breakdown voltage of the tube and went out when the voltage dropped to the extinction voltage. Rentschler (**2**) modified this method by using a cold cathode relay tube in place of the neon tube. The relay tube supplied sufficient current to operate a mechanical relay and thus a counter mechanism. This instrument has found its main use in measurements of ultra-violet radiation.

The recording instrument herein described requires a power pack for operation with ordinary 115 volts, 60 cycles A.C. supply. If a used radio is

[1] Contribution no. 12, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Northeastern States and the Electrical Engineering Department of the Pennsylvania State College, State College, Pennsylvania.

available its power pack, including transformer, rectifier tube and socket, choke coil, and condensers may be used. Most of these items will be in usable condition, except perhaps for the electrolytic condensers usually supplied. These, however, can be replaced with superior paper or oil-filled condensers of sufficient capacity at a cost of one to two dollars. If a used radio is not available the items required for the power supply may be purchased separately. The total cost of the power supply should not exceed five dollars ($5.00).

The wiring diagrams for both the power supply and integrating instrument are presented in figures 1 and 2.

In operation, light striking the light-sensitive element in the phototube causes a current to flow, which is very closely proportional to the intensity

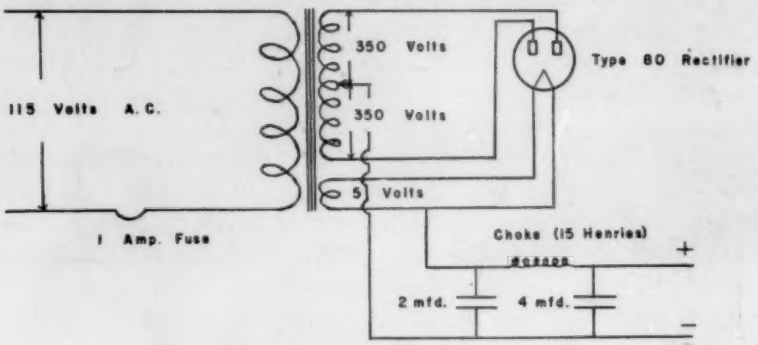

FIG. 1. Wiring diagram for power supply.

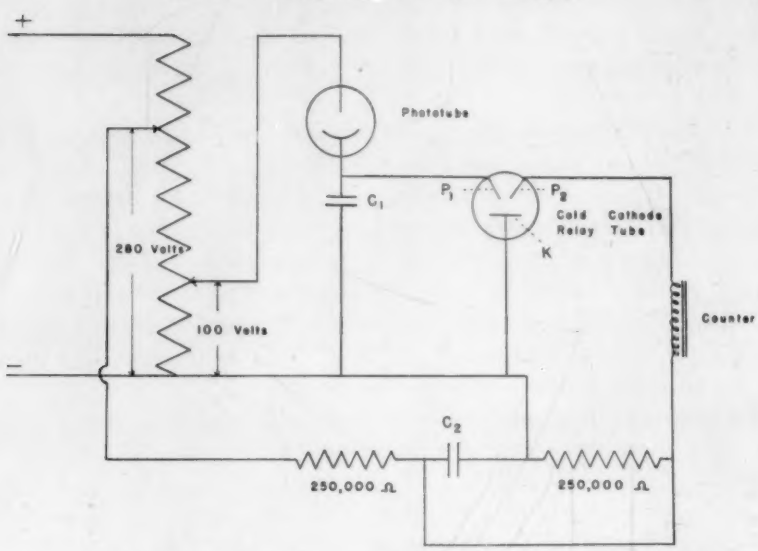

FIG. 2. Wiring diagram of integrating light recorder.

of the light. This current charges the condenser C_1. The relation between current, i (a function of time), and the potential difference, V, at time, t, across the condenser is:

$$V = \int_0^t idt + V_0$$

in which the potential is assumed to be V_0 at time $t = 0$. This potential difference is applied directly between the starting anode (P_1) and the cathode (K) of the cold cathode gas discharge tube (Western Electric type 313C). When the potential, V, reaches a critical value, the gas in the tube ionizes, allowing the condenser C_1 to discharge (at which time the potential returns to V_0) and at the same time discharging C_2. The discharge from this latter condenser passes through a sensitive magnetic telephone relay, pulling down the armature and actuating the counter. As soon as the discharge passes, the tube deionizes and the circuit returns to its initial condition. A very sensitive counter is required because the amount of current passing through the relay tube depends on the capacity of the condenser, C_2, which is limited not only by the current rating of the tube but also by the small allowable time delay in recharging the condenser, C_2, between successive operations of the counter.

A counter which has worked very satisfactorily was constructed from a dollar alarm clock. The balance wheel was removed and a 1/32-inch hole drilled in the escapement lever. One end of a fine brass spring wire was passed through this hole and the other end fastened to the armature of the sensitive telephone relay. The clock and relay were so mounted that the throw of the relay armature moved the escapement lever sufficiently to allow one tooth of the escapement wheel to pass, as would occur in the normal operation of the clock. In this counter, the gear mechanism of the clock is driven by the main spring while the magnetic relay supplies only the small force required to trip the escapement. The clock can be calibrated easily before the balance wheel is removed, by counting the number of escapements per minute in normal operation. With the cheaper clocks this usually is 120. Therefore, with such a counter, 7200 operations would be required for a complete revolution of the minute hand and 86,400 would represent a complete revolution of the hour hand. The regular face of the clock may be removed and a face reading in total number of discharges substituted, or, by proper calibration, the face may be calibrated to read directly in any convenient units.[2]

[2] Light has been defined as the radiant flux within the wave length limits of sight, evaluated according to its capacity to produce visual sensation. The foot-candle is the practical unit of illumination and represents the illumination on a surface, normal to the direction of the light, from a point source of one International Candle, one foot distant. Since the human eye is not equally sensitive to all regions of the visible spectrum and

The capacity of the condenser, C_1, which stores the charge depends on the current output of the phototube. Since there may be considerable difference in the sensitivity of photo-electric cells even of the same type and by the same manufacturer, it is necessary to determine the size of the condenser, C_1, experimentally. An intensity of 500 foot-candles incident on the particular General Electric PJ–22 phototube used resulted in about 50 discharges per minute when a 2.0 mfd. (microfarad) condenser was employed. Less frequent operation of the counter could be obtained by using a larger condenser but error owing to leakage in the condenser would have increased; more frequent operation through the use of a smaller condenser was undesirable owing to the failure of the counter to register accurately at speeds greater than 80 operations per minute. The use of a smaller condenser would be desirable if the instrument is designed to record lower maximum light intensities. The authors have experienced no difficulty in recording light intensities well below one foot candle with condenser capacities of the order of 0.01 mfd.

The instrument is adaptable to a number of uses, inasmuch as the phototube can be placed at any distance from the counter and several counters can be operated from one power supply unit. The maximum intensity striking the phototube should not exceed that recommended by the manufacturer, which in the case of the General Electric PJ–22 tube is 500 foot-candles. In this preliminary arrangement for the measurement of sunlight, the radiation is received on a horizontal surface, similar to Weather Bureau practice. The maximum intensity on the phototube is reduced to 500 foot-candles by means of two sheets of frosted opal glass. WALLACE (3), in a study of methods of sampling visible radiation, has indicated that a single light sensitive unit placed inside and at the base of an opal glass sphere is to be preferred as the receiver for radiation measurements to be used in physiological work. In any receiver care should be exercised to prevent direct sunlight from striking the phototube and to prevent the temperatures of the phototube from exceeding 50° C.

The linearity of the above instrument has been checked from 5 to 450 foot-candles incident on the phototube. A straight line relationship exists between the intensity of the light and the current output of the phototube when the quality of light is unchanged. A decided shift in the quality of light either to the blue or red end of the spectrum alters the current output of any of the phototubes now available. The General Electric PJ–22 tube

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since the mechanical equivalent of light varies with the wave length, it is not possible to convert foot-candles into energy units except for monochromatic light or for heterogeneous light of known and constant composition. For many applications, however, comparative data would be acceptable. For this purpose, the integrated light may be expressed in foot-candle hours which is the product of intensity and time.

is least sensitive to light of about 5000 Å wave length and increases in sensitivity to longer and shorter wave lengths (see fig. 3). For measuring

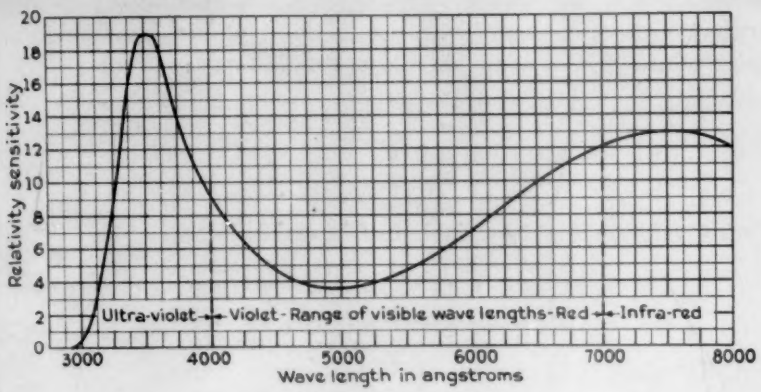

FIG. 3. Static sensitivity characteristic of the PJ–22 phototube. (Reproduced through the courtesy of the General Electric Company, Schenectady, N. Y.).

natural daylight, in which the quality does not vary greatly except at sunrise and sunset and where comparative data are primarily desired, this limitation of the phototube is not serious.

After assembly, the instrument was calibrated against a standardized Weston Model 603-foot-candle meter using sunlight at noon on a clear day as a light source. For comparative data the natural light available during any period may be expressed as foot-candle-hours which is the intensity in foot-candles multiplied by the time in hours. Inasmuch as this instrument is integrating, the average intensity will be available. In calibration of the above instrument it was found that an average sunlight intensity of 240 foot-candles passing through two frosted opal glasses before reaching the phototube produced one counter operation per minute. The number of foot-candle hours during any period is equivalent to the number of discharges divided by the number of discharges the counter would make at an average intensity of one foot-candle for one hour. In this calibration, at one foot-candle, there was 1/240 discharge per minute or 60/240 discharges per hour.

$$\text{Then total f.-c.-hours} = n \div \frac{60}{240}$$

$$= \frac{n \times 240}{60}$$

$$= 4n$$

where n = number of discharges during the period.

Example:

16,800 discharges on July 21 = 4 × 16,800 = 67,200 f.-c.-hr.

Several examples of the data obtained are presented here as an indication of the variation in daylight at State College, Pa., during the months the instrument has been in operation.

Daily values of light available in foot-candle-hours:

April	8,	28,705
April	9,	6,312
April	10,	14,578
April	11,	50,496
April	12,	25,549

Weekly values of light available in foot-candle-hours:

April	1– 7,	inclusive,	280,935
April	8–14,	inclusive,	172,228
April	15–21,	inclusive,	186,515
April	22–28,	inclusive,	245,116

Monthly values of light available in foot-candle-hours:

March	1–31,	inclusive,	678,351
April	1–30,	inclusive,	1,000,754
May	1–31,	inclusive,	1,087,771
June	1–30,	inclusive,	1,201,993
July	1–31,	inclusive,	1,507,462
August	1–31,	inclusive,	1,198,561

After five months of uninterrupted operation a new calibration was made and compared with the original. There was no appreciable change, indicating that in all probability the characteristics of the gas relay tube and the phototube change very slowly with age. The change in calibration with the fluctuations in supply line voltage usually encountered may be expected to be negligible. Operation of the relay tube and the characteristics of the condensers are affected only by larger fluctuations in ambient temperature than those normally encountered in a laboratory or a greenhouse. No great change in sensitivity of the phototube would be expected if the 50° C. maximum ambient temperature recommended by the manufacturer is not exceeded.

The upkeep of the instrument is very small, since no moving electrical contacts are used which might be oxidized, and no mechanical parts other than the magnetic relay armature and counter are employed. The cold cathode relay tube requires no attention and has an almost indefinite life if it is not overloaded. It would seem that the low original cost of the instrument described (less than $25 as compared to commercially available instruments ranging in price from $150 to $500), its low current consumption (about 10 to 15 watts), and its probable long life would put within the reach of those interested in light as it effects growth, a means of studying some of the factors not heretofore determined.

LITERATURE CITED

1. ANDERSON, J. S. Photoelectric cells and their applications. Physical and Optical Societies. London, 1930.
2. RENTSCHLER, H. C. An ultra-violet light meter. Trans. Amer. Inst. Electr. Eng. **49**: 576–578. 1930.
3. WALLACE, R. H. Methods of sampling visible radiation. Plant Physiol. **12**: 647–666. 1937.

PRODUCTION OF SOLUTES IN GROWING EPIDERMAL CELLS

WILLIAM A. BECK

(WITH ONE FIGURE)

Growth by cell enlargement, which is characteristic of plant cells, may be defined as the irreversible increment in the volume without appreciable increment in the mass of the protoplasm. Investigation of this kind of growth was given considerable impetus by URSPRUNG and BLUM (**12**) when they discovered that the turgor of young cells is minimum when the growth is maximum, and also by WENT (**14**) and his school when they proved that the growth-promoting substance which induces this kind of growth originates in the region of cell proliferation.

Although it appears evident that the growth-promoting substance must exercise its effect on the living protoplasm (*cf.* **10**) there is still some question about the effect produced directly or indirectly on the cell wall and the production of solute in the protoplasm which is osmotically active in the cell sap cavity.

KLEBS (**5**) concluded from his experiments that the protoplasm is directly responsible for cell enlargement, which is, of course, hardly tenable in view of the facts known to us at present. HEGLER, SACHS, DEVRIES (**3, 7, 13**) and others assumed that cell enlargement is directly due to the stretching of the wall, which itself is caused by the turgor. This view was decidedly disputed by SCHWENDENER and KRABE (**8**), who contended that the wall grows actively by a process of intussusception with the cooperation of the protoplasm. PFEFFER (**6**) explains how the growth of the wall may be the result of active and passive growth, with consequent changes in the elastic constants of the wall. The fact which URSPRUNG and BLUM established beyond a doubt, that the turgor is minimum in the most actively growing cells (**12**) makes it certain that the turgor plays at most a secondary rôle in the process of cell enlargement. It also makes it questionable if the solutes which are necessary to maintain the turgor are elaborated by the protoplasm to a degree which is commensurate with the growth of the cell.

It is the purpose of the present investigation to determine the amount of solute which is produced during the various stages of cell enlargement.

Material and methods

The enlargement of the cells in different phases of development was first determined with the aid of an eye-piece micrometer. Then the osmotic value at incipient plasmolysis and the degree of contraction in passing from the normal state to the state of incipient plasmolysis were determined for

the same phases according to the methods given by URSPRUNG (**11**). Knowing the volumes in the two states and the concentration at incipient plasmolysis, it became possible to calculate the concentration of the sap in the normal state according to the equation:

$$(1)\quad O_n/O_g = V_g/V_n \text{ or}$$
$$(2)\quad O_n = O_g \times V_g/V_n;$$

in which O_n is the concentration of the cell sap in the normal state; O_g, the concentration in the state of incipient plasmolysis; V_n, the volume of the cell in the normal state and V_g, the volume in the state of incipient plasmolysis (*cf.* **11**, p. 1111).

Since the amount of solute in a solution varies directly as the volume and directly as the concentration, the total amount of solute present in the normal cells may be expressed in arbitrary units as the product of the normal volume and the normal concentration. In our particular case it was found that the diameter of the cells remains practically constant during cell enlargement, so that it was sufficient to multiply the concentration by the one variable, namely the length, in order to obtain an adequate expression for the total amount of solute present expressed in microns × mol.

The epidermal cells of Helianthus seedlings were employed. As is well known, proliferation of cells occurs only within a very small zone of the hypocotyl (less than 1 mm. long) and then growth occurs by cell enlargement. Since the cells in the neighborhood of the stomatal complex are very irregular they were avoided and relatively long series of regular cells were preferred.

The region from the base of the cotyledons to a point 35 mm. beyond was investigated. For convenience it was divided into 7 zones, each 5 mm. long. Etiolated seedlings that were 90 hours old and from 45 to 50 mm. high, which were raised under controlled conditions (temperature 25° C. 90 per cent. relative humidity) in peat and sand, from seeds of the 1939 harvest of a special strain cultivated by the author for the last five years were employed. The individual differences in the plants were negligible.

The sizes in the cells were determined for three points in each zone, but the average osmotic value at incipient plasmolysis, and the change in volume from the normal state to the state of incipient plasmolysis were calculated as an average for the entire zone and expressed as effective at the mid-point of the zone. Each reading accepted at any point for concentration of the sap or the length of the cell is the average of 30 independent determinations.

Results and discussion

The results obtained are assembled in table I and the graphs (fig. 1) which show the variation in the length of the cells and the variation in the total amount of solute present in the cells as they grow older make the comparison of the results easy.

TABLE I

THE SIZE OF GROWING EPIDERMAL CELLS OF *Helianthus annuus* SEEDLINGS AND THE AMOUNT OF SOLUTE WHICH THEY CONTAIN

ZONE	DISTANCE FROM THE COTYLEDONS	AVERAGE LENGTH OF THE CELLS	AVERAGE EQUIVALENT CONCENTRATION OF THE SAP	TOTAL SOLUTE PRESENT IN ARBITRARY UNITS
	mm.	*microns*	*mol.* ($C_{12}H_{22}O_{11}$)	*microns* × *mol.*
	1.0	15.2		
1	2.5	25.6	0.270	7.18
	4.0	38.9		
	6.0	79.3		
2	7.5	96.1	0.248	23.90
	9.0	115.8		
	11.0	127.9		
3	12.5	144.3	0.243	34.60
	14.0	155.0		
	16.0	165.3		
4	17.5	173.2	0.228	39.60
	19.0	181.4		
	21.0	189.2		
5	22.5	203.6	0.225	44.90
	24.0	206.8		
	26.0	209.2		
6	27.5	212.8	0.226	48.00
	29.0	216.4		
	31.0	219.0		
7	32.5	221.4	0.226	50.00
	34.0	224.2		

The fact that the curves run almost parallel shows that the production of solute must be practically proportional to the increment in volume or to the growth by cell enlargement. Both curves are typical growth curves. They show an inflexion at a point corresponding to the point on the hypocotyl which is 6.5 mm. from the base of the cotyledons. This is evidently the point of maximum growth and production of solute. These results are in agreement with those published elsewhere (*cf.* 1) regarding the relative growth in the hypocotyl of seedlings which were strictly similar to the ones used in the present work. There it was shown that the most favorable zone for studying growth by cell enlargement is the zone between 4.65 and 6.4 mm. from the base of the cotyledons. There a measuring microscope was used and the hypocotyl was intact except for the markings. In the present experiment zone 3 still shows considerable growth and solute production, but in zone 4 a decided decrement of both rates is noted and in zone 7 the rates of both are almost 0. In other experiments which still await publication, employing strictly similar plants, the suction tension and the wall pressure and the turgor were determined for the same regions and it was

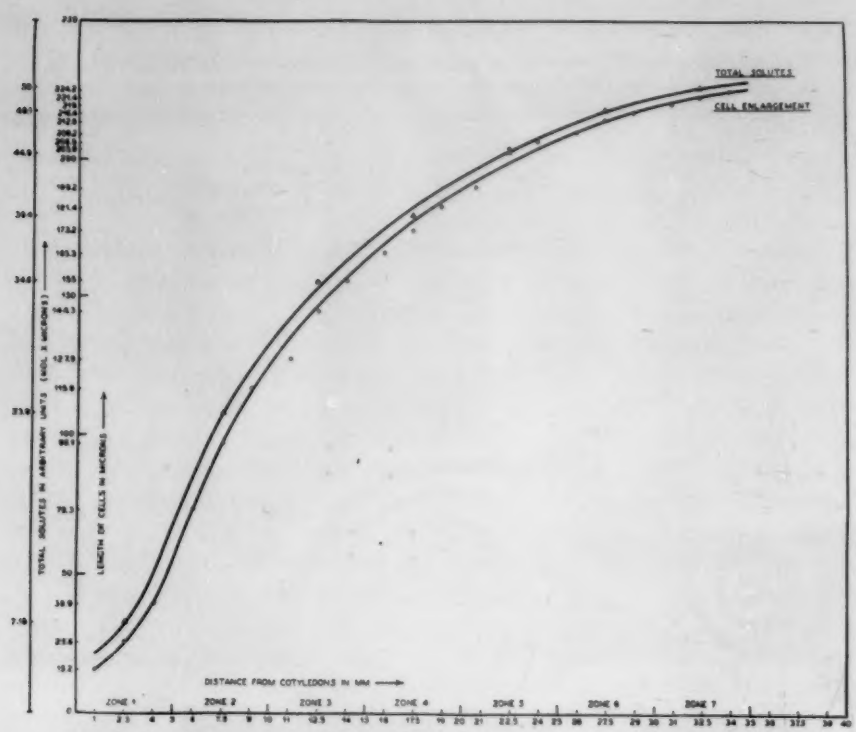

FIG. 1. Relationship between cell enlargement and total solutes of the cell sap.

found that the suction tension is highest in the growing region and lowest in the mature cells; the turgor and the wall pressure, however, were least in the growing region and began to increase decidedly in the mid-region of the fifth zone and were maximum in the mature cells where the suction tension was least. It is interesting to note that solute production declines with the increment of turgor and wall pressure, since it shows the increment in wall pressure must be caused by changes in the nature of the wall and not by increment in the production of solutes. Likewise the decline in the suction tension can hardly be attributed to considerable decrement in the rate of the production of solute but rather to increased counter pressure by the wall, which apparently has become more rigid. On the basis of this explanation it can be stated that the walls have become more rigid at a point 22.5 mm. from the base of the cotyledons. This result is in good agreement with that given elsewhere (**2**) regarding the critical point of the bending of hypocotyls in strictly similar plants, in response to gravity. There it was found that the regions which are farther removed than 25 mm. from the

base of the cotyledons hardly respond at all, and it was explained that the wall of the cells must be more rigid than in the younger cells.

These results are in agreement with the findings of URSPRUNG and BLUM (**12**) referred to above. They are also in agreement with the finding of SÖDING (**9**) who showed that the first phase of growth is irreversible and that the stretching of the wall by turgor must follow this phase, and that the wall must grow by intussusception. These results are furthermore in agreement with those of STRUGGER (**10**) who showed that growth by cell enlargement can hardly be attributed to the growth of the wall alone. He showed that, in the epidermal cells of Helianthus seedlings, the protoplasm is significantly more viscous in the younger cells than in the mature cells and proved the existence of a "protoplasmatic gradient."

Together with the results obtained by URSPRUNG and BLUM (**12**), SÖDING (**9**), STRUGGER (**10**), and HEYN (**4**), the results of this research make it apparent that the growth of the cell by cell enlargement is a physiological process, and that it cannot justly be attributed to the turgor alone, nor to the growth by intussusception in the wall alone, nor to the changes in the state of the protoplasm alone. The production of solutes and the increment in the mass of the wall, and the physical changes in the protoplasm must be regarded as phases of the growth process, or possibly only as concomitant circumstances; but any one alone is not the prime cause of growth.

Conclusions

From the given results it may be concluded that:

1. The production of solute in the growing epidermal cells of etiolated Helianthus seedlings is proportional to the growth of the cells.

2. The production of solute does not increase the turgor of the cells in proportion to the amount of solute produced, and it cannot be regarded as the primary cause of growth.

3. The production of solute certainly aids in maintaining the suction tension of the growing cells and thus insures sufficient water supply for the growing parts of the seedling. It also aids in maintaining sufficient turgor so that the young plastic wall does not collapse, and that growth in the wall by intussusception is facilitated.

Summary

A method is devised and described for determining the amount of solute produced in growing cells.

The enlargement of growing epidermal cells and the amount of solute produced in them are determined and correlated for etiolated Helianthus seedlings.

It is found that the amount of solute produced is proportional to the growth of the cells.

It is concluded that the production of solute facilitates the growth process in general, and the growth of the wall by intussusception in particular, but that it cannot legitimately be regarded as the prime cause of growth by cell enlargement.

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LITERATURE CITED

1. BECK, W. A., and DONNELLY, W. The Helianthus test. Studies Inst. Divi Thomae **2**: 179–189. 1939.
2. ———. Bending and cell enlargement in the hypocotyl of *Helianthus annuus*. Studies Inst. Divi Thomae **2**: 259–282. 1939.
3. HEGLER, R. Über den Einfluss des mechanischen Zug auf das Wachstum. Cohns Beitr. Biol. Pflanzen **6**: 383–432. 1893.
4. HEYN, A. N. J. Further investigations on the mechanism of cell elongation and the properties of the cell wall in connection with the elongation. Protoplasma **19**: 78–96. 1933.
5. KLEBS, B. Beiträge zur Physiologie der Pflanzenzelle. Unters. Bot. Inst. Tübingen **2**: 65. 1886.
6. PFEFFER, W. Pflanzenphysiologie vol. 2, in 2. Aufl. Engelmann, Leipzig. 1904.
7. SACHS, J. Über das Wachstum der Haupt- und Nebenwurzeln. Arb. Bot. Inst. Würzburg **1**: 385. 1874.
8. SCHWENDENER, S., and KRABE, G. Über die Beziehungen zwischen dem Mass der Turgordehnung und der Geschwindigkeit der Längenzunahme wachsender Organe. Jahrb. wiss. Bot. **25**: 323–369. 1893.
9. SÖDING, HANS. Über die Wachstumsmechanik der Haferkoleoptile. Jahrb. wiss. Bot. **79**: 231–256. 1933.
10. STRUGGER, SIEGFRIED. Beiträge zur Physiologie des Wachstums. I. Zur protoplasma-physiologischen Kausalanalyse des Streckungswachstums. Jahrb. wiss. Bot. **79**: 406–471. 1933–1934.
11. URSPRUNG, A. Die Messung der osmotischen Zustandsgrössen pflanzlicher Zellen und Gewebe. Handbuch der biologischen Arbeitsmethoden Abt. XI. Teil **4**: 1109–1572. 1937.
12. ———, and BLUM, G. Eine Methode zur Messung des Wand- und Turgordruckes der Zelle nebst Anwendungen. Jahrb. wiss. Bot. **63**: 1–110. 1924.
13. DE VRIES, H. Untersuchungen über die mechanischen Ursachen der Zellstreckung. Engelmann, Leipzig. 1877.
14. WENT, F. W. Wuchsstoff und Wachstum. Rec. trav. bot. néerl. **25**: 1–116. 1928.

AUTOMATIC CONDUCTIVITY MEASUREMENTS OF CO_2[1]

D. G. Clark, John Shafer, Jr., and O. F. Curtis

(WITH TWO FIGURES)

In research on photosynthesis it is often desirable or sometimes necessary to record automatically frequent measurements of the amount of carbon dioxide absorbed by a leaf or plant. By none of the older methods could carbon dioxide intake be determined over intervals shorter than one or two hours. The measuring of carbon dioxide by its effect on the conductivity of a sodium hydroxide solution is accurate and rapid, but has not previously been made automatic. The excellent spectrometric method of McAlister

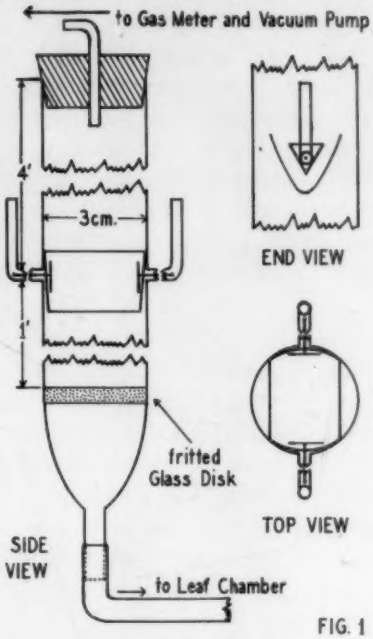

Fig. 1. Equipment for absorption of CO_2.

(**2**) could be made automatic, but the cost of the basic equipment is considerable. The method to be described in the present note seems to fill the need for rapid automatic recordings. It is based on the conductivity method employed by many other workers.

The arrangement of equipment can best be described under two headings, *viz.*, that used for gas flow and absorption and that for carbon dioxide measurements. The equipment involved in the absorption of carbon dioxide is

[1] The authors are indebted to Dr. A. Dounce of the Biochemistry Department at Cornell University and to the Laboratory Division of the Corning Glass Company, for aid in the construction of the absorption tower.

diagrammed in figure 1. The absorption tower[1] in figure 1 needs special description. This is an adaptation of the tower described by HEINICKE and HOFFMAN (1); its basal end contains a fritted glass disk which breaks up the air stream into small bubbles to insure complete absorption of the carbon dioxide. The bubbles rise through the sodium hydroxide solution, exerting a stirring action. Triangular platinum electrodes are fused in the tower a short distance below the level acquired by the liquid surface after the flow of air through the tower has attained equilibrium. The bubbles are diverted from the electrodes by a glass trough and pass to the top of the liquid, where they break. Part of the liquid draining back down the tower is caught in the trough and replaces that which has drained out at the bottom corners. If the trough is properly made the space between the electrodes will be filled with constantly changing, bubble-free sodium hydroxide solution. The solution in the trough does not change in composition as rapidly as that at the bottom of the tower, but the lag is essentially constant and, therefore, immaterial. Since the electrodes are separated by clear liquid, conductivity can be measured at any time, or even continuously. Consequently, automatic records are possible. This is in contrast to the usual conductivity techniques, which involve cessation of air flow and thorough mixing of the absorbing solution before conductivity measurements are made. With that sort of technique frequent automatic readings are impossible.

Since temperature has a marked effect on conductivity, the absorbing solution (NaOH) is kept in a controlled temperature room throughout, except for the brief period while it is being measured in an automatic pipette. By suitable manipulation of several stopcocks the sodium hydroxide solution is measured out and introduced into the absorption tower. Then air is drawn over the leaf, through the absorbent, and through the gas meter.

Naturally, the electrical circuits for automatically recording carbon dioxide should be designed to fit the particular requirements of the operator. The arrangement used by the authors (fig. 2) consists of three separate circuits, though two are in parallel with each other. One circuit contains the absorption and conductivity tower, wired as one arm of a Wheatstone bridge. The other three arms are built with fixed resistances. Accompanying the change from sodium hydroxide to sodium carbonate as carbon dioxide is absorbed, there is a corresponding decrease in the conductivity of the solution. If the initial sodium hydroxide solution is always of the same strength, and if such factors as temperature, electric current and the electrode system remain constant, then the degree of unbalance of the bridge is dependent on the amount of carbon dioxide which has been absorbed by the time of any particular reading. Such being the case, the amount of carbon dioxide absorbed can be measured by its effect on the amount of current going through the recording ammeter connecting the arms of the

Wheatstone bridge. It is believed that quicker readings are obtained by measuring the current across an unbalanced bridge than by balancing the bridge and recording the necessary resistance changes. It is believed, also, that the two methods give readings which are of about the same order of accuracy. In the present arrangement the current is recorded on an Esterline-Angus 5-mil recording ammeter. From experimental data, graphs can be constructed for converting the amperage values to carbon dioxide values. The current passed through the Wheatstone bridge must be of constant voltage. We are using 110-volt 60-cycle A.C. line current regulated by a Raytheon voltage regulator, with satisfactory results.

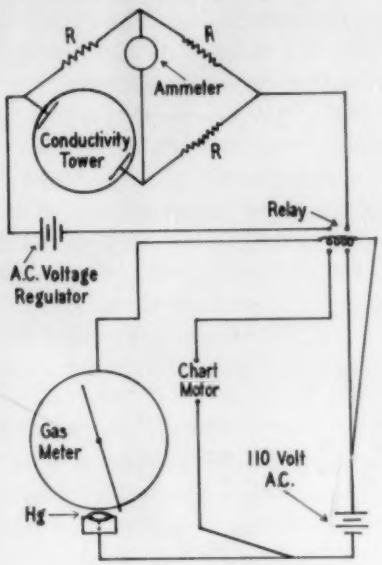

FIG. 2

FIG. 2. Wiring diagram for automatic measuring system.

The "chart motor" is the electric motor that winds the chart on the recording ammeter, and is an integral part of that instrument. It is wired in such a way as to run only while a recording is being made.

The third circuit is the "starter" for the other two. The indicator needle of the wet-test gas meter is extended to dip into a puddle of mercury at a given point of the needle's revolution, thus completing the circuit through the relay coil. The relay closes, thereby completing the other two circuits, and causing the conductivity of the tower solution to be recorded. By varying the number of arms on the meter needle, contact can be made after each complete revolution or after any desired fraction of a revolution. The conductivity record is, therefore, automatically a record of air volume. By having a constant rate of air flow, and by timing that rate at the start of a run, the readings can be converted to time intervals if desired.

At least two towers must be operated simultaneously, since carbon dioxide absorption or loss must be determined by difference. Several towers can be wired into the same electric circuit. The individual recordings require only three or four seconds each. The limiting factors as concerns the number of towers which can be used are: (1) the number of readings per revolution of gas meters; (2) the uniformity of the rate of air flow through the various meters. Unless all meters are running at identical rates, the recordings of two will sometimes coincide. This makes both readings uninterpretable. Obviously, the more recordings there are on the same ammeter in a given time, the more often will two readings coincide.

There are several sources of error which must be considered. Minor fluctuations in the mixing of the solution in a tower will produce minor variations in readings. If a different concentration of carbon dioxide is suddenly introduced, a short period (about 2 minutes) must be allowed for the establishment of the required new mixing gradient in the tower. In our experience, the most serious error is, unfortunately, not controllable: occasionally, the results obtained from one tower will not check with those obtained with replicate towers. As yet, no reason for such inconsistencies has been deduced. As a result of them no single run can be trusted; one must repeat the same experiment a number of different times. The standard deviation of the mean for an individual experiment is about $\pm$ 5 per cent. of the mean, when approximately 0.03 per cent. CO_2 is being absorbed.

Summary

There has been described a conductivity system for measuring photosynthesis. Readings are automatically recorded as frequently as desired. As used by the authors recordings are made every two minutes with an air flow of about 50 liters per hour. To achieve accuracy in conjunction with such rapid readings, very dilute hydroxide is being used (0.0013 N). This becomes exhausted in about 20 minutes when normal air is used. However, by changing the strength of hydroxide and the fixed resistances in the Wheatstone bridge, a longer run could be obtained with less frequent recordings. The outfit described should be easily adjustable to give a wide choice of frequencies of readings ranging from one or two minutes up to an hour or more, and should be adaptable to many different experiments.

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REFERENCES CITED

1. HEINICKE, A. J., and HOFFMAN, M. B. The rate of photosynthesis of apple leaves under natural conditions. Part I. Cornell Univ. Agr. Exp. Station Bull. 577. 1933.
2. MCALISTER, E. D. Time course of photosynthesis for a higher plant. Smithsonian Misc. Coll. 95: no. 24. 1937.

A HIGHLY SIMPLIFIED THERMIONIC CONTROL OF TEMPERATURE

RAYMOND H. WALLACE AND RALPH J. BUSHNELL

(WITH TWO FIGURES)

Three recent articles (**1, 2, 3**) describe methods of heat control which seem unnecessarily complicated in view of the present technical development of control tubes in the radio industry. Those methods, just mentioned, use mechanical relays and have wiring schemes which, although not complicated to those acquainted with radio, are nevertheless too difficult for many people to construct. One of the simplest and most effective schemes involving the use of thyratron tubes and no mechanical relays was described by SCHWENK and NOBLE (**4**) in 1937. The scheme here described is a further simplification and adaptation of thyratron methods.

Recently two well-controlled incubators were needed and were obtained by converting two old ovens that had long been around the laboratory unused. These ovens had been controlled by the usual mechanical thermostat and allowed a temperature variation of $\pm 2°$ C. The conversion of the ovens to valuable incubators was done as follows:[1] A five-volt transformer was mounted on top of the oven with the primary connected to the 110-volt line and the secondary connected to the two large prongs of a four-prong socket as filament supply for the control tube. Then the two heavy wires (the 110-volt line for the heater) that had led to the mechanical thermostat were disconnected and one of them soldered to the two small prongs (cathode and shield grid) of the four-prong socket. The other wire was soldered to a clip to *attach to the tip (anode) of the control tube.* The tube is now wired with plate and filament voltage and all that remains is to connect to the new thermostat. Any type of thermostat can be used. For the ovens just mentioned, coiled bimetallic thermostats were taken out of ten-cent store thermometers. The thermostat was left mounted on the metallic dial of the thermometer, but the hand was broken off and a short shaft soldered in its place. An insulated contact was mounted so the free end of the thermostat could touch it. The thermostat was then mounted inside the oven with the shaft sticking through the top of the oven. An ordinary dial was attached to the shaft. Adjustment for any range is obtained by turning this dial which winds up or unwinds the bimetallic thermostat. The wiring is completed by attaching one contact of the thermostat directly to the control grid, while the other one is connected to the anode through a 10-megohm resistance.

[1] Full-sized blue prints giving all details of construction are available for 25 cents.

It takes about four hours to convert an oven, and the chamber one obtains will maintain temperature to $\pm 0.1°$ C. as measured by mercury thermometers and to $\pm 0.25°$ C. as measured by a recording potentiometer and thermocouple. To get these good values it is, of course, necessary to circulate the air with a small fan.

The tube used in this scheme is the General Electric mercury vapor thyratron, F.G. 95, which carries 2.5 amperes at 1000 volts, but differs from the tube used by SCHWENK and NOBLE (**4**) in that it has a 5-volt, 4.5-ampere filament and is designed to activate on extremely small grid currents. The tube is so constructed that it will not fire until a plus charge is put on the control grid. When the grid is connected to the anode by the way of the thermostat and grid leak as described above, the tube fires on each plus cycle of the A.C. current and the chamber heater is in action.

The tube maintains a delicate control of the heater, which is very desirable. When the thermostat is well off balance, the tube activates strongly and the heater may draw its full amperage. On nearing its balance, however, the current drops sharply and hovers almost continuously around 0.05 to 0.1 amperes. The tube functions as a variable resistance and consequently the heater never gets hot and is never cold. This eliminates the "all-on," "all-off" condition which is always present in mechanical relays. It likewise makes unnecessary the light-bulb shunt across the heater circuit which SCHWENK and NOBLE used. Measurements with a recorder and a thermocouple showed the floor temperature is as constant as the air temperature being maintained, and is only 1° C. above it. This constant temperature of the heater under continuous load is the most important characteristic of this scheme of temperature control. It makes possible the construction of chambers with little or no stratification even without mechanical stirring. To do this one needs only to embed low-wattage heaters in the six walls of the chamber and connect them all in series with the control tube. The control is obvious at all times by the flicking of the tube, and its continuous glow when the full current is flowing.

It is often necessary in controlling temperature to activate the heater, the reverse of that above, with bimetallic thermostats. Thus, with mercury thermostats, one wants the heater to cut out when contact is made. This can be done very simply with the scheme used here. In figures 1 and 2, the solid lines show the wiring thus used with bimetallic thermostats. If one moves the thermostat from position A and places it in position B, that is, if it is moved from being in series between the anode and the control grid, and is placed in series between cathode and control grid, the tube activates while the thermostat is open but ceases to fire when it is closed. In making this change it will be noted that the 10-megohm leak remains in place between anode and grid while a 500,000-ohm grid leak is added along

with the thermostat between the grid and the cathode. When the thermostat is closed the grid is at the potential of the cathode and the tube does not fire; when the thermostat is open, the grid is at the potential of the anode and the tube fires on each plus half of the cycle as before.

One of us (**5**) has used the F.G. 95 as control tube in recording potentiometers since 1934, and found them very dependable. Although some of them have had more than three years continuous use, none has burned out in service. They are fragile and, therefore, frequently damaged in shipment. If, however, they function after being heated up a few times, they

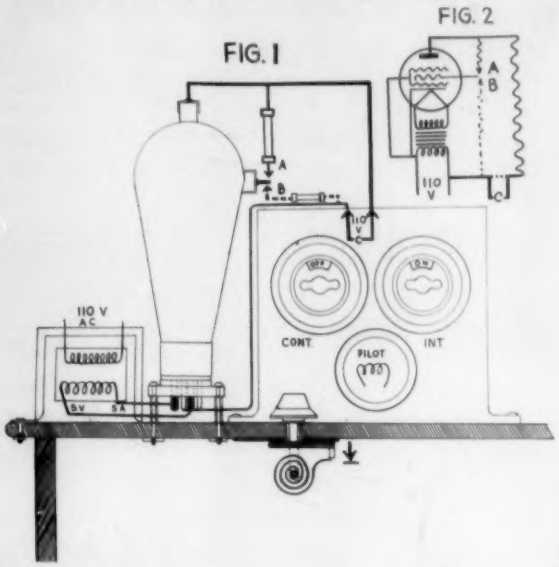

FIG. 1. Pictorial diagram showing connections and spacing of parts; A, position of thermoregulator when it is a bimetallic thermostat; B, position of thermoregulator when it is a mercury thermostat. When A is used, B is left entirely out; but when B is used, the grid leak at A is connected to grid. C is position of usual mechanical thermostat and, if connection is made at the arrow points, the wiring of switches and pilot bulb remain unchanged.

FIG. 2. Schematic diagram showing complete wiring. A, B, and C, the same as in figure 1.

are undamaged and give no trouble subsequently. The total cost of material for converting an oven is less than $15.00; $12.00 for the tube, and $2.00 for the transformer.

The tube activates on from 1 to 1/10 microamperes, so no appreciable current passes through the thermostat contacts. This small current necessitates good insulation in the grid circuit for otherwise, leakages may activate the tube.

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LITERATURE CITED

1. HALL, A. C., and HEIDT, L. J. An electronic relay for heat control. Science n.s. **92**: 133–134. 1940.
2. HAWES, R. C. A high-performance electronic relay. Ind. Eng. Chem. Anal. Ed. **11**: 222–223. 1939.
3. ————————. An electronic relay with improved characteristics. Science n.s. **93**: 24. 1941.
4. SCHWENK, H. S., and NOBLE, D. E. A relay-free mercury vapor triode control for a constant-temperature water bath. Jour. Phys. Chem. **41**: 809–810. 1937.
5. WALLACE, R. H. A recording potentiometer for use in physiological investigations. Plant Physiol. **12**: 487–498. 1937.

BRIEF PAPERS

SPECTROSCOPIC ANALYSIS OF PLANT EXTRACTS FOR CHLOROPHYLLS *a* AND *b*[1]

C. L. Comar and F. P. Zscheile

Absorption coefficients for chlorophylls *a* and *b* in ether solution have been determined recently on preparations of high purity.[2] The quantitative application of absorption data to simple plant extracts is the ultimate criterion both for the identity of the standard chlorophyll components with those in the extracts and for the accuracy of the standard absorption coefficients.

It is now possible to analyze plant tissues satisfactorily for total chlorophyll and for percentage of components *a* and *b*. The method is as follows: A weighed sample (about 10 gm.) of fresh leaf tissue is disintegrated in a Waring Blendor in the presence of acetone to which a small amount of calcium carbonate has been added. The resulting mixture is filtered by suction and the pigments are transferred to diethyl ether (Dupont technical, allowed to stand over solid NaOH and distilled). All the acetone is removed from the ether by repeated scrubbing through distilled water and the final solution is dried with sodium sulphate. If care is taken, emulsion formation can be avoided and quantitative results readily obtained. The absorption measurements are made at 6600 and 6425 Å. The spectral region isolated should not exceed 30 Å and large amounts of scattered radiation must be avoided. Other wavelengths may be used when the carotenoid content is not too high.

The absorption coefficients listed above are averages obtained from several pure chlorophyll preparations. Chlorophylls *a* and *b* may be determined most satisfactorily at wavelengths 6600 and 6425 Å. Total chlorophyll may be determined at intersection points of the absorption curves for components *a* and *b*. Component composition may be determined also at wavelengths 6130, 5890, and 5460 Å.

Sample 1 was a spinach extract from which all carotenoids had been removed by washing with methanol, followed by precipitation of the chlorophylls and final removal of carotenoids by washing with petroleum ether.[2] Samples 2 and 3 were aliquots of sample 1 to which pure *beta*-carotene had been added. In these three samples, the *a* to *b* ratio does not represent the natural ratio in the leaf because of preferential loss of *b* during the methanol

[1] Supported by the Herman Frasch Foundation for research in agricultural chemistry, paper no. 213.

[2] Zscheile, F. P., and Comar, C. L. Bot. Gaz. 102: 463–481. 1941.

TABLE I

ANALYSIS FOR TOTAL CHLOROPHYLL AND PERCENTAGE COMPOSITION

WAVE-LENGTH	SPECIFIC ABSORPTION COEFFICIENTS *a*	*b*	SAMPLE 1 CHLOROPHYLL *a* + *b* (CAROTENOIDS REMOVED)	SAMPLE 2 CHLOROPHYLL *a* + *b* + 2 MG. CAROTENE PER 100 ML.	SAMPLE 3 CHLOROPHYLL *a* + *b* + 6.2 MG. CAROTENE PER 100 ML.	SAMPLE 4 SPINACH LEAF EXTRACT
6600	102.0	4.5	* 80.3 per cent. *a*	79.4 per cent. *a*	79.2 per cent. *a*	71.1 per cent. *a*
6425	16.3	57.5	0.0606 gm./l.	0.0590 gm./l.	0.0597 gm./l.	0.0418 gm./l.
6000†	9.95	9.95	0.0600	0.0604	0.0623	0.0422
5810†	8.05	8.05	0.0602	0.0603	0.0622	0.0418
5680†	7.11	7.11	0.0610	0.0618	0.0665	0.0414
6130‡	15.6	8.05	79.8 per cent. *a*	80.1 per cent. *a*	83.4 per cent. *a*	70.4 per cent. *a*
5890‡	5.90	10.3	78.9 per cent.	77.3 per cent.	72.7 per cent.	66.6 per cent.
5460‡	3.03	6.24	78.2 per cent.	64.8 per cent.	38.6 per cent.	64.5 per cent.

* These values calculated from simultaneous equations.

† Crossing points on absorption curves.

‡ Calculated from absorption at this wavelength and average of values of total concentration which were unaffected by the presence of carotenoids.

washing. Sample 4 was an extract made by the analytical procedure described in paragraph 2.

The following conclusions are evident from the analytical results:

1. All wavelengths listed give correct results when carotenoids are absent.
2. Analytical values at 5890 and 5460 Å are extremely sensitive to the presence of carotenoids, due to the nature of the curves. Even at 6000 Å, carotenoids may cause appreciable error, as shown by sample 3, which has an extremely high carotene content. Note that at 6600 and 6425 Å, the values of sample 3 agree with those of sample 1.
3. The wavelengths, other than 6600 and 6425 Å, that can be used depend upon the carotenoid content of the leaf.

This analysis of spinach is representative of many similar determinations. Broccoli tips and leaves of corn, sweet potato, barley, and tomato have also been analyzed satisfactorily. A more detailed discussion of the chemical precautions and instrumental conditions essential for quantitative results will be presented in another paper.

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PHOTOSYNTHESIS WITH RADIOACTIVE CARBON, AND THE DISTRIBUTION OF THE INTERMEDIATE PRODUCTS IN THE PLANT CELL

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Recent experiments with radioactive carbon (C^{11}) have shown that green plants take up CO_2 reversibly in the dark (**2, 3, 5**). The evidence accumulated thus far indicates the presence of high molecular weight compounds which bind the CO_2 in a combination stable in boiling concentrated hydrochloric acid.

In this note experiments are described in which the distribution of the initial photosynthetic products within Nitella cells have been investigated. Radioactive carbon was used as a tracer according to the technique described by RUBEN, KAMEN, and HASSID (**3**).

Experimentation

Nitella plants were used in this work, since these plants release their cell contents very readily on slight crushing. Since no grinding was necessary, it was possible to obtain a maximum of intact chloroplasts by crushing the cells in a 0.5-M glucose solution (**1**). The cytoplasm and vacuolar sap were separated from the chloroplasts and other bodies by centrifuging. Microscopic investigation of the chloroplasts showed that the majority of them were left intact after this treatment.

1. Intact Nitella plants were exposed to $\overset{*}{C}O_2$ for 25 minutes both in the light and in the dark. At the end of this period the plants were removed from the reaction flasks and immediately crushed in 0.5-M glucose. The cell materials were then separated as stated and their activity tested. Each sample was boiled with 12-N hydrochloric acid, in order to expel any $+ 4\ \overset{*}{C}$ present. After several washings of the cell wall material with water and of the chloroplasts with 0.5-M glucose, no activity was found either in the chloroplasts or the cell wall material from the plants which were kept in the dark during the exposure to $\overset{*}{C}O_2$. The aqueous, colorless solution obtained from centrifuging of the crushed plant material contained 90 per cent. of the total activity after the first centrifugation, two further washings of the residue removed almost all of the activity from the water-insoluble material. In contrast, the plants which concurrently were kept in the light during this period contained nearly four times as much activity in their chloroplasts as in the non-chloroplast material. This activity in the chloroplasts could not be removed by further washings with 0.5-M glucose.

2. In another series of experiments, Nitella plants were crushed immediately before exposure to C^*O_2. This material was filtered through cheese cloth, transferred to Warburg vessels and exposed to C^*O_2 for 25 minutes in the dark and in the light. No uptake of C^*O_2 was observed either by the cytoplasm and vacuolar sap or by the chloroplasts in a form resistant to boiling hydrochloric acid.

3. Intact Nitella cells were exposed to C^*O_2 in the dark for 25 minutes and then crushed also in the dark in 0.5-M glucose. Intact cells were filtered out. The suspension was exposed to light for 20 minutes. No decrease in the activity of the aqueous fraction was observed and no activity was found in the chloroplasts.

Thus it appears that intact plant cells are a prerequisite not only for this particular dark reduction of CO_2 but also for the further reduction of the carboxyl group formed in the dark (**4**). Whatever compound reduces CO_2, whether in the light or in the dark, it seems evident that it is non-chloroplastic, or only weakly adsorbed on the chloroplasts and easily removed by physical injury to the living cell.

The writer is indebted to Dr. S. Ruben and Dr. M. D. Kamen for the supply of radioactive CO_2, as well as for their advice and criticism, and to Dr. G. Mackinney for his helpful suggestions.

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LITERATURE CITED

1. Granick, S. Quantitative isolation of chloroplasts from higher plants. Amer. Jour. Bot. **25**: 558–561. 1938.
2. McAlister, E. D. The chlorophyll-carbon dioxide ratio during photosynthesis. Jour. Gen. Physiol. **22**: 613–636. 1939.
3. Ruben, S., and Kamen, M. D. IV. Molecular weight of the intermediate products and a tentative theory of photosynthesis. Jour. Amer. Chem. Soc. **62**: 3451–3455. 1940.
4. ————, ————, and Hassid, W. Z. Photosynthesis with radioactive carbon. II. Chemical properties of the intermediates. Jour. Amer. Chem. Soc. **62**: 3443–3450. 1940.
5. Smith, J. H. C., and Cowie, D. B. Absorption and utilization of radioactive carbon dioxide by sunflower leaves. Plant Physiol. **16**: 257–271. 1941.

NOTES

Annual Election.—The eighteenth annual election of the American Society of Plant Physiologists was held during May and June, 1941. The secretary-treasurer, Dr. W. E. Loomis, Department of Botany, The Iowa State College, has announced the results of the election as follows:

President, Dr. Edwin C. Miller, Kansas State College.
Vice-president, Dr. W. E. Loomis, Iowa State College.
Secretary-treasurer, Dr. P. J. Kramer, Duke University.
Executive committee, Dr. W. F. Loehwing, University of Iowa.
Editorial committee, Dr. Otis F. Curtis, Cornell University.

The new officers become active on July 1, 1941. The Dallas meeting will be the most important immediate concern of these officials. They desire, and will need, hearty cooperation in planning and executing the necessary work incident to the eighteenth annual meeting and program. Early replies with titles, and aid wherever it is requested are always appreciated during this busiest season of the year.

Reports of other matters voted upon in the 1941 election will be made in the October number of Plant Physiology.

Purdue Section.—The Purdue Section of the American Society of Plant Physiologists reports a very successful season during the year 1940–1941 under the chairmanship of Dr. R. E. Girton, of the School of Science, Purdue University. Fifteen regular meetings were held during the year, with an average attendance of 25. Two of these were dinner meetings. Dr. F. G. Gustafson, Botany Department, The University of Michigan, addressed the Section at its first dinner meeting in October, on the subject: *Hormones in Relation to Plant Growth.* At the last dinner meeting of the year in May, Mr. Glenn M. Smith of the Purdue University Agricultural Experiment Station presented his important work on *Sweet Corn Breeding.*

The new officers elected by the Purdue Section for the year 1941–1942 are: Chairman, Dr. F. P. Zscheile, of the Department of Agricultural Chemistry, School of Agriculture; and secretary-treasurer, Dr. W. R. Mullison, of the Department of Biology, School of Science.

Vacation.—The editor-in-chief will be out of residence from Chicago during the entire summer, until about September 15. Correspondents will please note that, because of rapid movement from place to place, the editor will find it difficult to answer communications promptly. Papers offered for publication should be sent to the office of the secretary of the editorial committee, Dr. W. F. Loehwing, Iowa City, Iowa, as usual, and other matters allowed to wait until September.

Winthrop John Vanleuven Osterhout.—It is with a great deal of pleasure that we are able to present in this number two fine portraits of Dr. OSTERHOUT, to whom this number of PLANT PHYSIOLOGY is dedicated in honor of his approaching 70th birthday anniversary, which occurs on August 2, 1941. A brief biography was published in PLANT PHYSIOLOGY for January, 1940, at the time he was elected to a CHARLES REID BARNES life membership in the American Society of Plant Physiologists. On behalf of all members of the Society, we extend our best wishes and congratulations to him for the happy occasion of his anniversary, and for a long period of pleasurable activity following his retirement from formal service. He has earned it by nearly fifty years of pioneering work which has advanced our understanding of many physiological processes.

Minor Elements.—The second annual supplement to the third edition of the *Bibliography of References to the Literature on the Minor Elements and their Relation to Plant and Animal Nutrition* has been issued by the Chilean Nitrate Educational Bureau, HERBERT C. BREWER, Director, 120 Broadway, New York. It contains 67 pages, in two-column format, with three indexes, one by elements, one by botanical common names, and one by authors. About 50 elements are included, and 150 crop plants. Slightly less than 400 authors are listed. The annotated bibliography is extremely valuable, and all students of plant nutrition will be grateful for the continuation of this service to research. Members of the Society should obtain a copy of the supplement by writing to Mr. BREWER.

Hunger Signs in Crops.—Interest in the problems of plant nutrition, intimately bound up with animal nutrition, has grown rapidly in recent years. The American Society of Agronomy, in cooperation with the National Fertilizer Association, has published a symposium on *Hunger Signs in Crops*. It is a very attractive monograph, with about 80 plates in colors, and a few less than 100 text figures. The chapters deal with crops, or groups of crop plants, with titles and authors as follows: Why do plants starve? by GEORGE D. SCARSETH and ROBERT M. SALTER; plant-nutrient deficiency in tobacco, by J. E. MCMURTREY, JR.; deficiency symptoms of corn and small grains, by GEORGE N. HOFFER; plant-nutrient deficiency symptoms in the potato, by H. A. JONES and B. E. BROWN; plant-nutrient deficiency symptoms in cotton, by H. P. COOPER; plant-nutrient deficiencies in vegetable or truck-crop plants, by J. J. SKINNER; nutrient-deficiency symptoms in deciduous fruits, by O. W. DAVIDSON; plant-nutrient deficiency symptoms in legumes, by E. E. DETURK; and symptoms of citrus malnutrition, by A. F. CAMP, H. D. CHAPMAN, GEORGE M. BAHRT, and E. R. PARKER.

It is a very valuable survey because it brings a large section of our

knowledge of mineral deficiencies into a single work. Agricultural agents, teachers, and extension men will find it particularly helpful. And many research men will find it handy to use in comparing their own observations. It is written in popular style, but does not sacrifice accuracy in bringing the information to a popular level.

It is printed by Judd and Detweiler, Washington, D. C., at the modest price of $2.50 per copy. The press work is admirable. We recommend this book as one which should be in the hands of all plant physiologists.

Enzyme Research.—The great work by BAMANN and MYRBÄCK, *Die Methoden der Fermentforschung*, continues to come from the press of Georg Thieme, Leipzig. Lieferung 6 contains 336 pages, and Lieferung 7, 416 pages, ending on page 2588. Lieferung 6 continues with the hydrolases, specifically, with the carbohydrases, some of which were presented in Lieferung 5. The phyto- and zoo-amylases, fructanase, glucanases, cytases, and polyuronidases complete the list of carbohydrases. Then follow the nucleases, amidases, proteases, thrombase and blood coagulation enzymes, and oxynitrilese finish out the great group of hydrolases.

The last part of Lieferung 6 begins consideration of the desmolases and enzymes of biological oxidation and reduction. The first part of this section takes up alcoholic fermentation, normal fermentation by living yeasts and zymase preparations.

Lieferung 7 continues with the enzymes of oxydoreduction, glycolytic enzymes, dehydrases, cytochromes, and the oxytropic dehydrases. Section II of the desmolases is a short chapter on the oxyhydrases, such as glucose-oxyhydrase, ascorbic acid oxydase, dioxymaleic acid oxydase, oxalooxydase, amino acid oxydases, and aminooxyhydrases (tyraminoxydase, adrenalin oxydase, aminoxydase, histaminase, and diaminoxydase).

Following this section, the enzymes of aerobic respiration are taken up, the true oxydases. The fourth section takes up the enzymes that make the first attack upon the sugar molecule (hexosephosphorylase); section V, the true desmolases (aldolase, carboxylase, amino acid decarboxylases, etc.); section VI, enolase; and section VII, the hydratases and related enzymes.

Each section is written by some expert in the special field of which he writes, or thoroughly familiar with the progress in methods of attack. It is impossible in a short review to do justice to this outstanding compilation. It deserves a place in the libraries of all institutions where enzyme research is in progress.

The prices stated for these two Lieferungen are: Lieferung 6, RM 25.20; Lieferung 7, RM 31.20. These are the Auslands price quoted by the publisher. It should be remembered that, for obvious reasons, the work is sold only in complete sets.

Modern Fruit Production.—An excellent work by two outstanding horticulturists, Dr. J. H. GOURLEY, and Dr. FREEMAN S. HOWLETT, both of the Ohio Agricultural Experiment Station, and The Ohio State University. The publishers are the Macmillan Co., New York, and the price quoted is $4.50 per copy. It is a book of 579 pages.

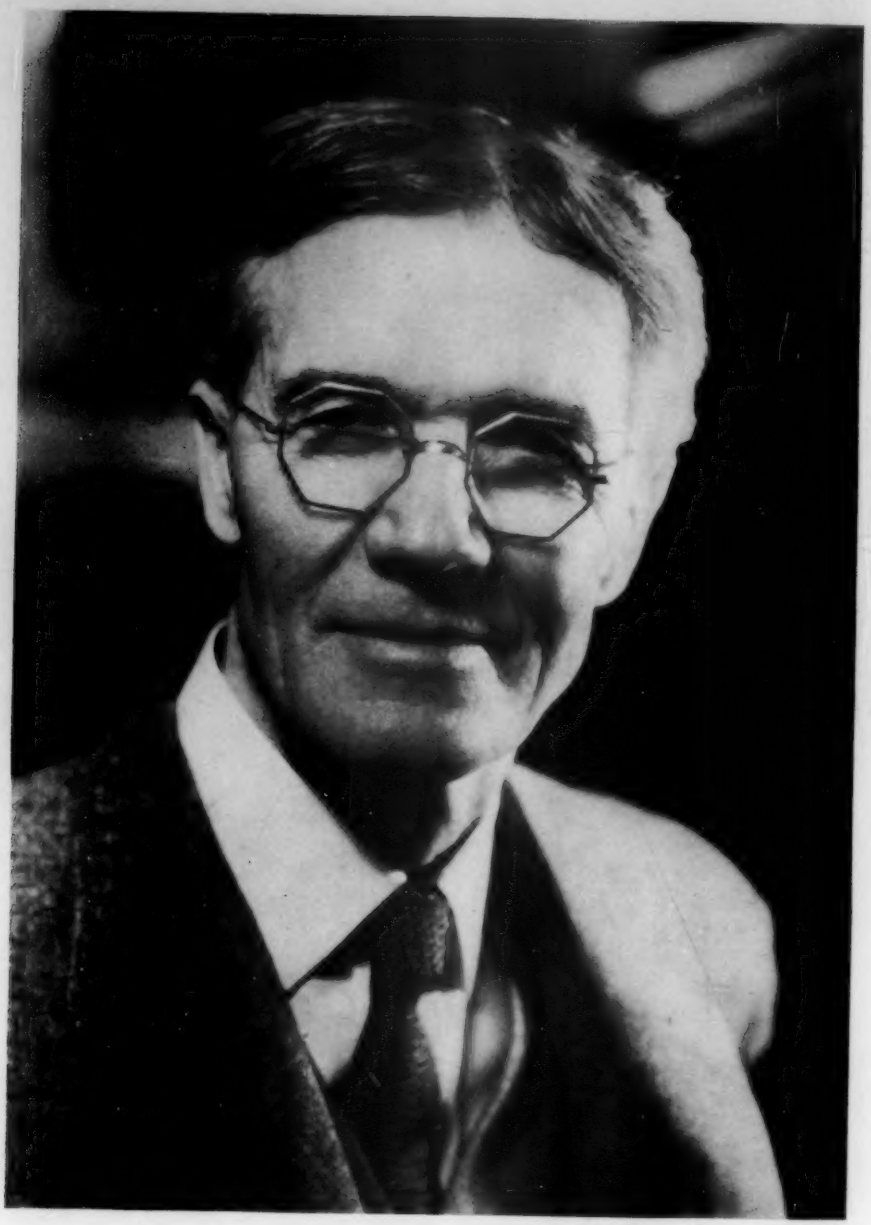
There are 17 chapters, several of which are introductory. The story of the fruit industry is told in the first chapter, and the fruit plant and its parts are described in the second. The subsequent chapters are given the following titles: Factors affecting flower formation; site and soil for the fruit plantation; laying out and planting the orchard; cultural practices; fertilizers and manures for the orchard; water relations of fruit plants; pruning of fruit plants; fruit setting; fruit thinning and alternate bearing; the handling and storage of fruit; winter injury; nutrient deficiencies and disorders; propagation and stocks; the origin and improvement of fruits; and orchard, vineyard, and small fruit costs.

The volume represents a complete rewriting and expansion of the previously published *Textbook of Pomology*. It is intended as a practical guide to fruit production, but has included enough of the modern results of botanical and physiological research to make it an up-to-date guide. It is a very clear presentation, and will be a welcome addition to the literature of horticulture.

Annual Review of Physiology.—The third Annual Review of Physiology has been issued by Annual Reviews, Inc., Stanford University, California. It contains 26 reviews, as follows: The relation of bioelectric potentials to cell functioning, by G. H. BISHOP; the physiological effects of radiant energy, by H. LAURENS; physiological aspects of genetics, by A. H. STURTEVANT; developmental physiology, by E. WITSCHI; growth, by C. E. PALMER and A. CIOCCO; temperature regulation, by J. C. SCOTT and H. C. BAZETT; energy metabolism, by T. M. CARPENTER; respiration, by F. C. SCHMIDT and J. H. COMROE, Jr.; physical properties of protoplasm, by E. F. ADOLPH; muscle, by W. O. FENN; the digestive system, by J. E. THOMAS; liver and bile, by W. B. HAWKINS; formed elements of the blood, by G. M. HIGGINS; heart, by C. J. WIGGERS and H. D. GREEN; peripheral circulation, by V. E. HALL; electrical activity of the brain, by H. H. JASPER; the autonomic nervous system, by D. SHEEHAN; the special senses, hearing, visual receptors, and vibratory sensations and pain, by E. BARANYM, R. GRANIT, and Y. ZOTTERMAN, respectively; physiological psychology, by H. S. LIDDELL; kidney, by L. LEITER; metabolic functions of the endocrine glands, by S. SOSKIN; endocrine aspects of the physiology of reproduction, by O. RIDDLE; reproduction in mammals, by M. H. FRIEDMAN; bacterial chemotherapy, by E. K. MARSHALL, Jr.; histamine and anaphylaxis, by W. FELDBERG; and exercise, by A. H. STEINHAUS.

These reviews have been prepared with great care. They portray the recent advances in physiology with clarity and accuracy, and are of the utmost importance to intelligent research in the field of physiology. The price of this volume is $5.00, as usual, and there is no better bargain, anywhere, in the field of physiological literature. The war in Europe has interfered with the receipt of foreign journals, and with the preparation of reviews by foreign experts. Nevertheless, many of the reviews are critical summaries, guiding the reader in appraisal of the progress, others a synoptic review of what has been accomplished. Naturally one prefers the reviews which offer appraisal and criticisms of the investigations, since this is the essence of progress, but all will be found valuable to the working physiologists.

The American Physiological Society, Annual Reviews, Inc., the capable editors, Dr. JAMES MURRAY LUCK and Dr. VICTOR E. HALL, and the collaborators who performed the valuable services in preparing the reviews deserve, and will receive, the thanks and enduring gratitude of all workers who benefit from their efforts. Those who desire to own a copy, should address Annual Reviews, Inc., Stanford University, California.



ALEXANDER PIERCE ANDERSON
NOVEMBER 22, 1862

UNIV. OF MICH.

THIS NUMBER OF PLANT PHYSIOLOGY

IS DEDICATED TO

ALEXANDER PIERCE ANDERSON

IN CELEBRATION OF

HIS EIGHTIETH BIRTHDAY

NOVEMBER 22, 1941